VWR
We Enable Science

Issue 33
September 2014

VWR bioMarket™

The market source for **Life Science!**

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CELL BIOLOGY

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SUPPORTING PRODUCTS

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CORNING

Falcon® Cell Culture Multi-Flask

An easier, more productive way to culture cells

- Even distribution of media across all layers for homogeneous cell populations.
- Ability to mix in the Falcon Multi-Flask saves time and reduces risk of contamination.
- Flexible design lets you pour or aspirate/recover cells using a pipette.
- Consistent surface treatments for predictable scale-up.
- Manufactured in compliance to cGMP standards.

Falcon Cell Culture Multi-Flasks enable you to grow more cells faster and easier, thereby making your cell culture workflow more productive.
The tissue culture-treated Falcon Multi-Flasks are available in 3- or 5-layer formats that provide 525 cm^2 or 875 cm^2 cell growth surface area. They can be used with a wide range of liquid volumes (up to 50 ml/layer).

Designed to fit your protocol

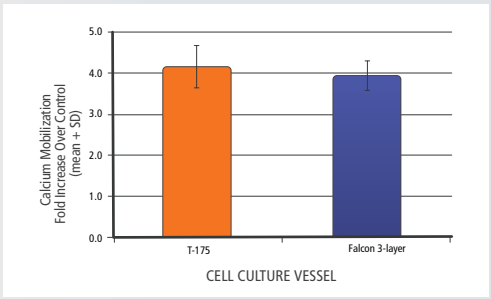
Falcon Multi-Flasks, offer the same footprint, the same reagent volumes and the same cell seeding densities as 175 cm^2. flasks. Plus, you'll be using the same proven surface treatment as all other Falcon flasks.
Improves your cell culture productivity
Falcon Multi-Flasks deliver a thoughtful design that simplifies your workflow. You can eliminate multiple steps and reduce the risk of contamination.

Pipette access allows you to aspirate media to exchange with fresh media and recover cells without pouring.
The mixing port allows rapid mixing within the Falcon Multi-Flask to create your cell suspension, transfect your cells, or add other reagents directly to the flask. The port also provides for a uniform distribution of media and cells to facilitate homogeneous cell growth on all layers.

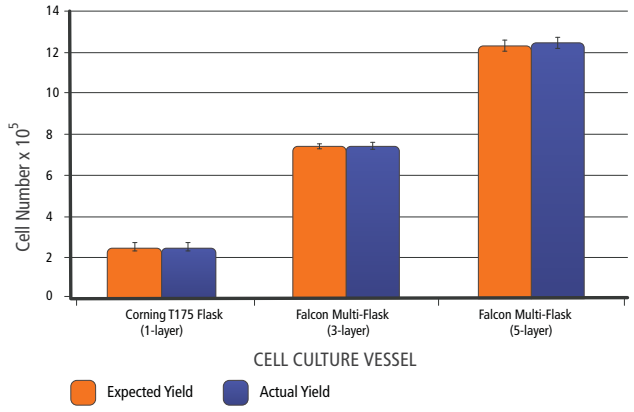
More consistent cell growth

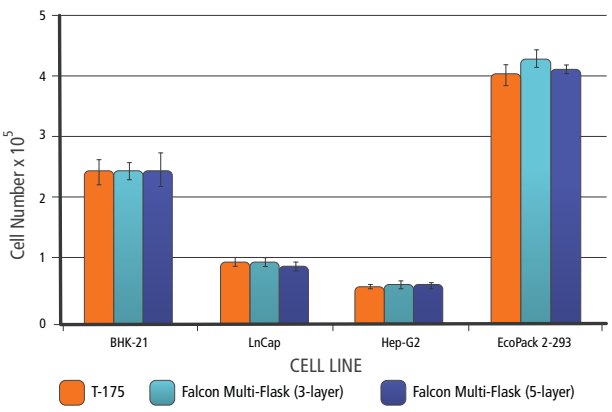
Falcon Multi-Flasks' even distribution of media, proven vacuum-gas tissue culture surface treatment, and effective gas exchange all combine to provide an optimal cell culture environment. The result is higher cell yield and a more homogeneous cell population.
Visit www.corning.com/lifesciences for more information.

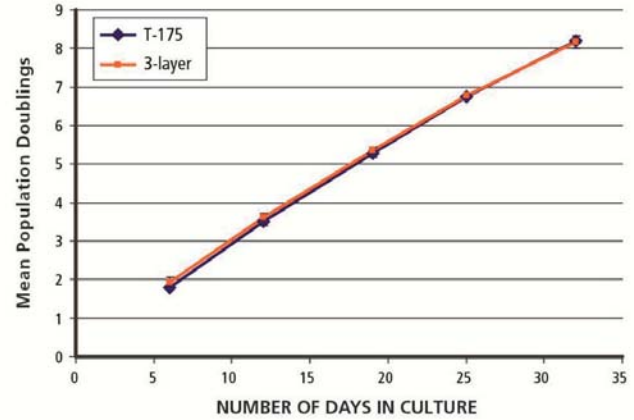
Consistent cell growth
This figure illustrates homogeneous cell growth between layers of Falcon® Multi-Flasks. BHK-21 cells grown to >80% confluence in 3-layer Falcon Multi-Flasks and control T-175 flasks were fixed and stained with crystal violet. Control flasks and individual layers of the multi-flask vessels were cut and scanned.



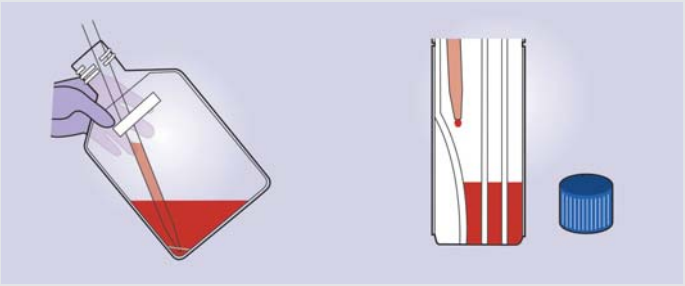
Consistent cell physiology
CHO-M1 cells were grown in a T-175 and a 3-layer Falcon Multi-Flask for 72 hours. Cells were harvested and re-seeded on 96-well PDL plates for GPCR Calcium mobilisation assay. Cells were challenged with M1 agonist, Carbachol (300 µM) and responses were recorded 1 minute following compound addition. Data is expressed as fold-increase in calcium flux compared to control cells challenged with assay buffer (no drug). No significant difference in agonist response was observed between cells harvested from either vessel type. Data shown is representative of three independent experiments®.





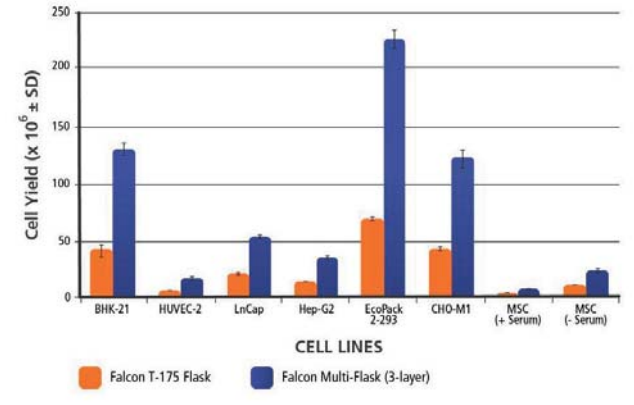


Predictable scale up
Three and five times more BHK-21 cells were grown and recovered from 3- and 5-layer Falcon® Multi-Flasks than T-175 flasks (left). Cell yields per cm2 were equivalent in 3- and 5-layer Falcon Multi-Flasks and T-175 flasks for BHK-21, LnCap, Hep-G2, and EcoPack™2-293 cells (right).



Ideal for pluripotent stem cells
Human Mesenchymal Stem Cells were cultured in MSCGM™ medium (Lonza) and seeded at 4000 to 5000 cells/cm2 in a T-175 Falcon Flask and a 3-layer Falcon Multi-Flask. The cells were cultured until they reached 80% confluence (~6 days). Cells were harvested, counted and cumulative population doubling of hMSCs from each vessel type were calculated over five consecutive passages. Immunophenotypic properties of hMSCs grown in the Falcon Multi-Flask as determined by flow cytometric analysis (FACS) confirmed that hMSCs were negative for surface markers CD14, CD34, CD45, CD79a, and HLA-DR and positive for expression of surface markers CD73, CD90, and CD105. Respective isotype controls for each marker antibody are shown in grey.

Simplified workflow
- Pipette access eliminates the need for pouring. It also facilitates the use of a wide range of liquid volumes (up to 50 ml/layer), efficient recovery of valuable cells and reagents and minimises the risk of contamination.
- Mixing port eliminates mixing outside the vessel, saving you time and effort. This unique in-vessel mixing allows for rapid and uniform distribution of cells and reagents.
- Compatibility with similar volumes of reagent usage and cell seeding densities as T-175 flasks makes scaling up a familiar and simple process.



Product specifications	
Working volume range	>5 ml per layer for dissociating
	<50 ml per layer for cell expansion
Moulded in graduations	0 to 50 ml per layer in 10 ml increments
Graduation accuracy	10%
Cap vent membrane	0.2 µm hydrophobic membrane
Cell growth surface	Tissue culture treated, optically clear

Falcon Cell Culture Multi-Flask		
Description	**Pk**	**Cat. No.**
3-layer, 525 cm² tissue culture treated	12	734-2456
5-layer, 875 cm² tissue culture treated	8	734-2457
3-layer, 525 cm² ECM Mimetic Fibronectin peptide	8	734-2696
5-layer, 875 cm² ECM Mimetic Fibronectin peptide	6	734-2697
3-layer, 525 cm² ECM Mimetic Collagen I peptide	8	734-2698
5-layer, 875 cm² ECM Mimetic Collagen I peptide	6	734-2699

Compatible with your cell lines
Diverse cell lines and primary cultures (with and without serum) can be grown and efficiently recovered from Falcon Multi-Flasks. Expected yield was determined using average cell yield from control T-175 flasks multiplied by three and five times for the 3- and 5-layer Falcon Multi-Flasks, respectively.

Falcon® Cell Culture Multi-Flask
An easier, more productive way to culture cells

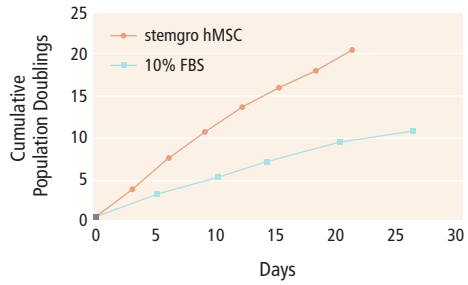
CORNING

Corning® stemgro® hMSC Medium

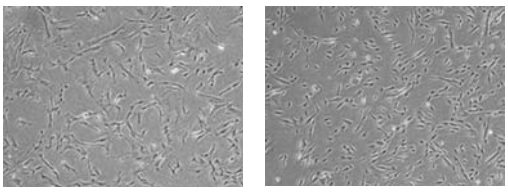
Serum-free, defined medium for efficient expansion of hMSCs on Corning CellBIND® Surfaces

- Complete solution - medium, supplement, surface
- Serum-free, xeno-free, defined formulation
- Superior cell performance relative to 10% FBS
  - Higher cell yield in shorter time
  - Shorter and more consistent cell doubling time
- No need for surface coating
- Cells retain normal immunophenotype and karyotype after multiple passages
- Cells retain trilineage differentiation potential after multiple passages

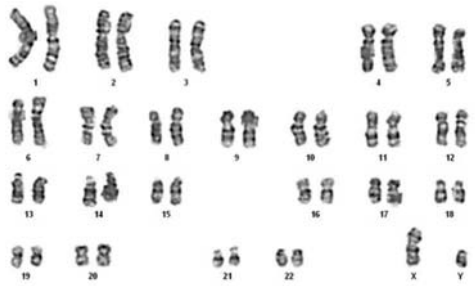
Corning stemgro hMSC Medium has been designed for the maximum expansion of human mesenchymal stem cells (hMSCs) derived from bone marrow, cord blood, or adipose tissue. stemgro hMSC Medium is chemically defined as its components are either chemically synthesised or recombinantly produced and purified. stemgro hMSC Medium offers the convenience of no vessel coating with biological materials if used with Corning CellBIND® Surface cell culture vessels, saving the cost of coating materials and time needed for coating.



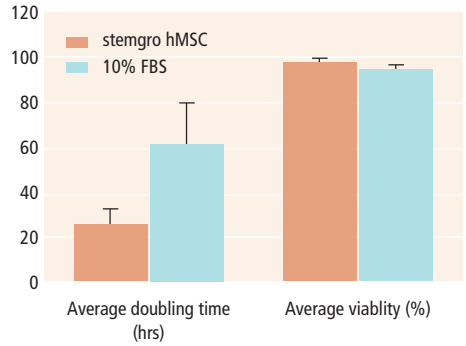
Two-fold higher population doublings in stemgro hMSC Medium compared to 10% FBS



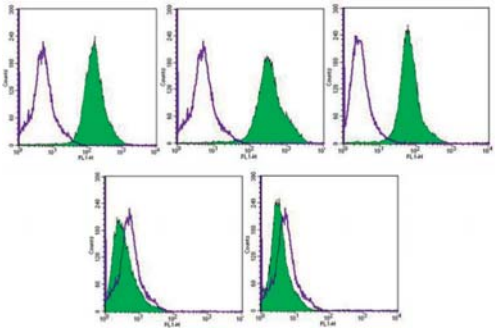
Typical hMSC morphology after multiple passages in stemgro hMSC Medium



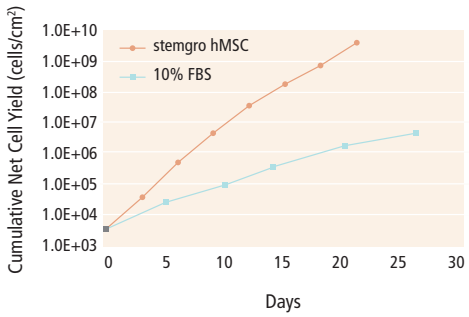
Normal karotype after 7 passages in stemgro hMSC Medium



Consistent doubling time and high viability across 7 passages in stemgro hMSC Medium



Normal immunophenotype after 5 passages in stemgro hMSC Medium



From 4000 to 4 billion cells in 20 days in stemgro hMSC Medium

Adipogenic

Chondrogenic

Osteogenic

Trilineage differentiation after 7 passages in stemgro hMSC Medium

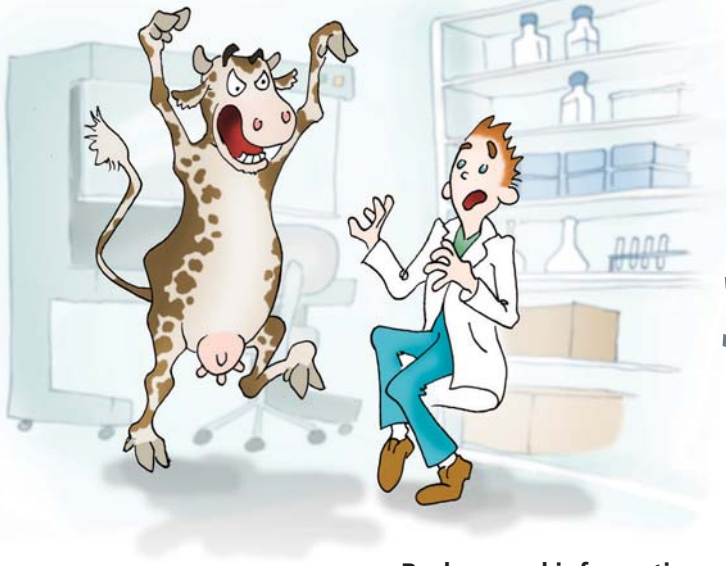
Corning CellBIND® Surface, with vent cap, sterile	Pk	Cat. No.
25 cm²	200	734-0090
75 cm²	100	734-0091
150 cm²	50	734-0092
225 cm²	25	734-0094

At Corning, cells are in our culture. In our continuous efforts to improve efficiencies and develop new tools and technologies for life science researchers, we have scientists working in Corning R&D labs across the globe, doing what you do every day. From seeding starter cultures to expanding cells for assays, our technical experts understand your challenges and your increased need for more reliable cells and cellular material.
It is this expertise, plus a 160 year history of Corning innovation and manufacturing excellence, that puts us in a unique position to offer a beginning-to-end portfolio of high quality, reliable cell culture consumables.

Corning CellBIND® flasks	Pk	Pk	Cat. No.
6 well plate, Corning CellBIND® Surface clear, with lid, sterile	5/bag	50	734-1210

	Size (ml)	Pk	Cat. No.
stemgro Basal Medium	450	1	40-410-KIT
stemgro Supplement	50		

Mad cow disease is not in the lab



BSE and FBS 30 years later

With the occurrence of BSE in the 1980´s, many regulations have been established to reduce the risk of bovine derived products in human consumption and other applications. These regulations have strongly influenced the preferences and selection of FBS in cell cultures. A lot of scientific work has been done in this field and today, much more is understood about the occurrence and transmission of this disease.

Background information

When BSE was first discovered in the UK in 1986[1], little was known about the cause of the disease or how it was transmitted. As a result, countries throughout the world responded by banning the importation of all bovine products, including blood products, from affected countries. After the discovery of the prion by Stanley Prusiner[2] as the causative agent of BSE, and his report of how it is spread by the feeding of Meat and Bone Meal (MBM) to cattle, the World Animal Health Organization (OIE), and regulatory authorities throughout the world, established standards banning the feeding of mammalian MBM to cattle and adopted slaughter practices that minimise the risk of BSE transmission.

Thanks to all these efforts, the rate of BSE cases has fallen dramatically, from 37 280 cases in 1992 alone in the U.K., to only 7 cases worldwide in 2013, two of which were atypical or spontaneous BSE cases[3].

In spite of the dramatic reduction (almost elimination) of BSE cases in the world, and cattle exporting countries having implemented proper risk reduction procedures, many importing countries still maintained disproportionate BSE related bans on bovine blood and blood by-products.

BSE regulations

In 2011 the European Union recommended the use of the OIE classification[4] to replace the former GBR risk class system[5]. Presently, there are 25 countries in the "negligible risk level", among them Australia, Brazil, Chile, Colombia, Denmark, New Zealand, Panama, Paraguay, USA, and Uruguay [see table 1[4]].

The European Union states that blood is safe when coming from "negligible BSE risk" and "controlled BSE risk" origins[6].

Since 1998 the EU has had a mandatory BSE monitoring programme in place - ID passports for all animals and BSE testing for cattle covering 100% of animals in pre-defined risk groups[7]. The traceability system is also useful for the tracking of other animal diseases and making EU origin the preferred choice in some applications.

Anyway, for Fetal Bovine Serum (FBS), the BSE risk has for many years been known to be ZERO, regardless of origin! The incubation time for BSE is recognised to be several years; which has been taken into consideration in the EU BSE control programme, initially covering animals older than 24 months[7]. Additionally, studies have shown that BSE from infected mother cows is not transmitted to the offspring[8].

BIOWEST THE SERUM SPECIALIST!

Blood and blood by-products are exempt from BSE restrictions

At the beginning of the BSE outbreak, the understanding of the disease was low, and consequently meat and blood products were handled under the same regulations and restrictions, some of which are still in place ever since then.

Based on the improved knowledge of the questions surrounding BSE, in 2006 the OIE updated the Terrestrial Animal Health Code standards for BSE and clarified that: "Blood and blood by-products should not be subject to any importation restrictions relating to BSE, regardless of the BSE status of the exporting country, except that the cattle being slaughtered were not subjected to a stunning process, prior to slaughter, with a device injecting compressed air or gas into the cranial cavity, or to a pitching process"[9] and that: "blood and milk are not considered to play a role in the transmission of BSE"[10].

Regulations for Serum usage follow these developments

Other authorities follow the OIE recommendation and have adapted their regulations as well. The latest version of the 9 CFR 95.12 states that blood and blood products derived from bovines must come from animals that were "not subjected to a pithing process or to a stunning process with a device injecting compressed air or gas into the cranial cavity". This is the only USDA restriction relating to BSE for blood and blood products derived from bovines.

Conclusion

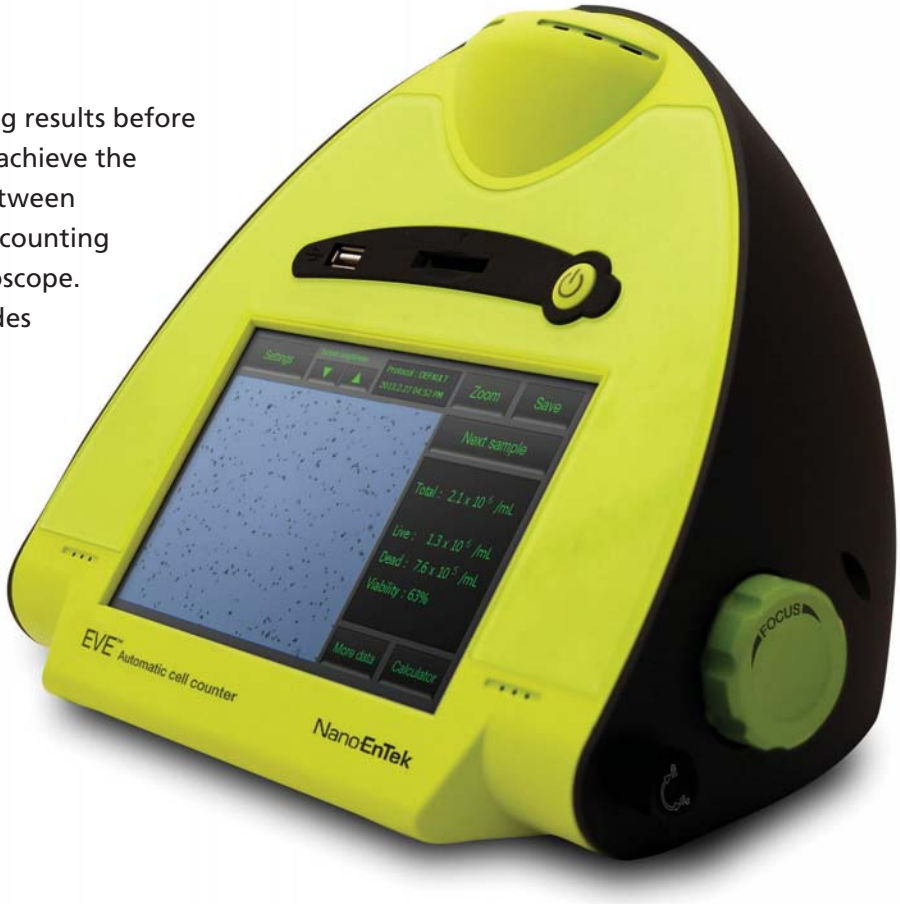
According to the OIE and other regulatory entities in the USA and EU, it can be concluded that BSE is irrelevant when selecting origins of bovine serum. MAD COW DISEASE IS OUT OF THE LAB – actually, it was never there in the first place!

Countries recognised as having a negligible BSE risk in accordance with Chapter 11.5 of the Terrestrial Code.			
Argentina	Denmark	Netherlands	Slovenia
Australia	Finland	New Zealand	Sweden
Austria	Iceland	Norway	USA
Belgium	India	Panama	Uruguay
Brazil	Israel	Paraguay	
Chile	Italy	Peru	
Colombia	Japan	Singapore	

1. *http://bmb.oxfordjournals.org/content/66/1/185.full.pdf+html*
2. *http://www.nobelprize.org/nobel_prizes/medicine/laureates/1997/press.html*
3. *http://www.oie.int/animal-health-in-the-world/bse-specific-data/*
4. *http://www.oie.int/en/animal-health-in-the-world/official-disease-status/bse/list-of-bse-risk-status/*
5. *http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2011:073:0001:0018:EN:PDF*
6. *http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003700.pdf; chapter 3.2.1.1. Bovine materials*
7. *http://ec.europa.eu/food/food/biosafety/tse_bse/monitoring_en.htm*
8. *Wrathall AE1, Brown KF, Sayers AR, Wells GA, Simmons MM, Farrelly SS, Bellerby P, Squirrell J, Spencer YI, Wells M, Stack MJ, Bastiman B, Pullar D, Scatcherd J, Heasman L, Parker J, Hannam DA, Helliwell DW, Chree A, Fraser H.: Studies of embryo transfer from cattle clinically affected by bovine spongiform encephalopathy (BSE). In: Vet Rec. 2002 Mar 23;150(12):365-78. PMID:11936410*
9. *http://www.oie.int/fileadmin/Home/eng/Health_standards/tahc/2010/en_chapitre_1.11.5.htm*
10. *OIE Terrestrial Animal Health Code Chapter 11.5. Article 11.5.27*
11. *http://www.gpo.gov/fdsys/pkg/FR-2013-12-04/html/2013-28228.htm;*

Comparison of cell counting methods between the EVE™ Automatic cell counter and a hemocytomer

Many researchers need to check counting results before each experiment. It is always helpful to achieve the standardisation of cell concentration between samples. Most common method for cell counting is using a hemocytometer under a microscope. The EVE™ Automatic cell counter provides accurate counting results with low variation in less time. This application note compares the EVE™ Automatic cell counter, to a conventional hemocytometer counting method.



EVE™ Automatic cell counter

The EVE™ Automatic cell counter uses state-of-the-art optics and provides image analysis to automatic cell counting. The EVE™ is a bench top cell counter designed to measure cell count and viability (live, dead and total cells) fast and precisely, using the standard trypan blue technique (Figure 1).

Using the same amount of sample that you currently use with the hemocytometer, the EVE™ takes less than 20 seconds per sample for a typical cell count and is compatible with a wide variety of eukaryotic cells plus provides information on cell size.

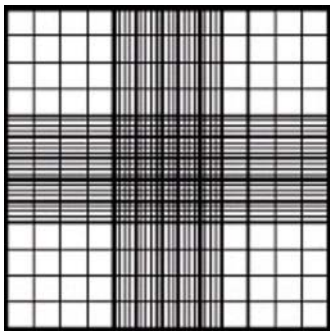
The EVE™ offers an intuitive user interface and support with the option to save and print cell count data using the EVE™ Software and USB drive, which is supplied with the instrument or available separately.
(For detailed information, please visit our website; http://www.nanoentek.com)

Hemocytometer, manual cell counting method

The hemocytometer is used for manual cell counting. It consists of 9 large squares, each measuring 1x1 mm, giving a total area of 3 x 3 mm of counting area. The volume of the chamber is 0.1 µm of each 1 x 1 mm square, giving a total volume for each.

Figure 1. Automatic cell counter, EVE™
Uses a disposable chamber that contains two enclosed chambers can count the cells with just 3 steps. Step 1. Load the sample, step 2, adjust focus, step 3. Obtain the results.

NanoEnTek



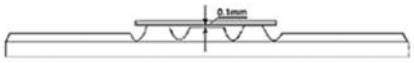


Figure 2. Hemocytometer, manual cell counting method.

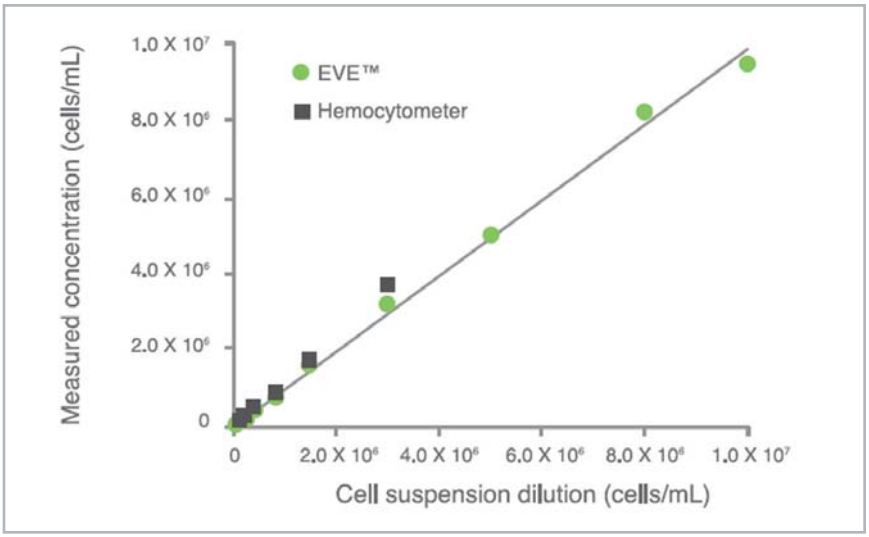


Figure 3. Measuring from the EVE™ extends further along the high concentration range compared to the hemocytometer readings. The EVE™ Automatic Cell Counter has a significantly higher effective concentration range than the hemocytometer.

Cell Type	Animal	Organ	Growth Properties
HeLa	Human	Skin	Adherent
NIH-3T3	Mouse	Embryo	Adherent
U-2 OS	Human	Bone	Adherent
Jurkat	Human	Blood	Suspension
KG-1	Human	Blood	Suspension
HepG2	Human	Liver	Adherent
Hep3B	Human	Liver	Adherent
LNCaP	Human	Prostate	Adherent
SH-SY5Y	Human	Brain	Adherent
SCN2.2	Rat	Brain	Adherent
F9	Mouse	Embryo	Adherent
MCF7	Human	Breast	Adherent
A549	Human	Lung	Adherent
GH3	Rat	Pituitary gland	Adherent

Sample preparation

To compare total cell counting result between the EVE™ Automatic cell counter and traditional hemocytometer, HeLa cell line was used. Cells were grown in defined medium in humidified 5% CO_2 incubator at 37°C. Cells were trypsinised using standard methods to prepare cell suspensions for counting. The cell samples were prepared in the range of 1 x 10^4 ml ~ 1 x 10^7 ml.

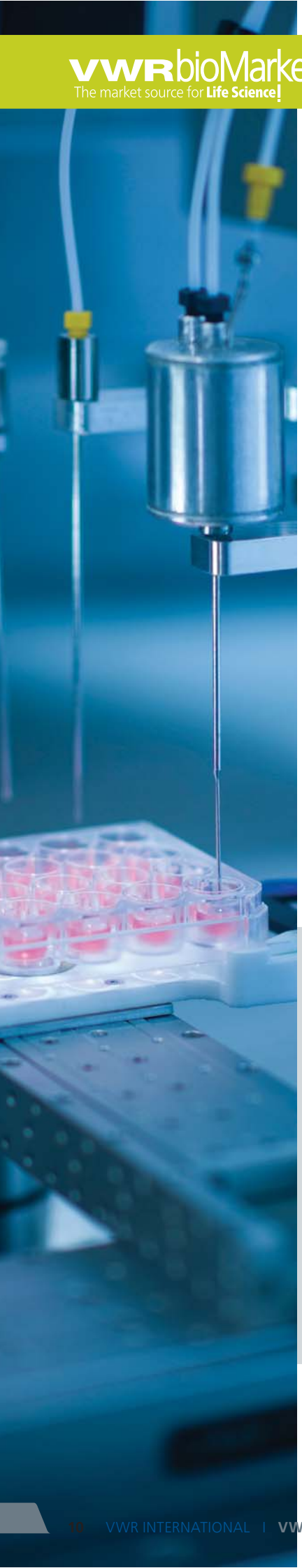
10 µl of the sample and the 0.4% trypan blue stain were mixed well using a pipette. 10 µl of the sample mixture was loaded on EVE™ Cell counting slide and hemocytometer.

Result

The results show that the glass hemocytometer counting method was less accurate than EVE™ Automatic cell counter. Moreover, the EVE™ Automatic cell counter counted a broader range of cell concentration than the hemocytometer. It also significantly extended further along the high concentration range than the hemocytometer readings.

In conclusion, EVE™ Automatic cell counter is the more efficient automatic cell counter for a broad range of cells. It not only reduces error from human variation in comparison with the traditional counting method, but also provides accurate and fast counting result through counting software and enclosed disposable chamber. Therefore, EVE™ Automatic cell counter ideal for various research applications in which cell counting and viability test are required.

Description	Contents	Cat. No.
Eve™ Automatic cell counter	Main device, power supply, USB stick, Eve cell counting slide, 50 slides (100 counts) with 1.5 ml of trypan blue (0.4%)	734-2675
Eve™ cell counting slide	50 slides (100 counts) with 1.5 ml of trypan blue (0.4%)	734-2676
Eve™ cell counting slide	1000 slides (2000 counts) with 1.5 ml of trypan blue (0.4%)	734-2677
Eve™ cell counting slide	5000 slides (10 000 counts) with 1.5 ml of trypan blue (0.4%)	734-2678
Test beads	1 ml	734-2674



Reconstruction of human epidermis using BRAND*plates*® Insert System

Author: Lena Schober, Andrea Traube; Fraunhofer
Fraunhofer-Institut für Produktionstechnik und Automatisierung IPA,
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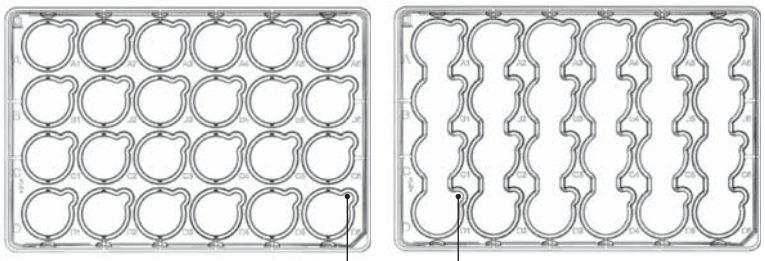
In the past decade new cell and tissue culture technologies have been generated to follow the European legislative that restricts animal experiments to a minimum. Particularly, the improvement of culture conditions for reconstructed human full thickness skin and epidermis equivalents based on cell culture inserts, lead to a successful commercialisation of these models. Today, with the help of artificial human tissues pharmaceutical and cosmetic industry carry out tolerance, toxicology and irritation studies day by day. In spite of every progress made in terms of media compositions and supplements, set-up and handling of organotypic cultures still needs a lot of time and expertise.

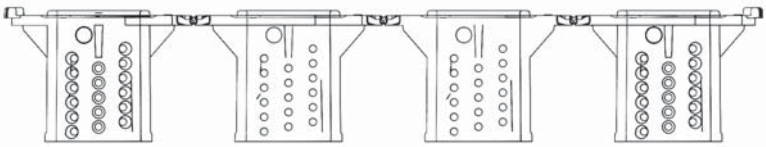
Up to now, increased hands on time and human-induced variations in culture processes can cut the success in high throughput reconstruction of human tissue. To deplete sources of unintended process fluctuations and to ensure high quality and reproducibility of in vitro tissues, the Fraunhofer Society and BRAND GMBH + CO KG collaborated in the development of the BRAND*plates*® Insert System.

This 24-well platform is especially customised to meet all requirements for a totally automated handling of insert-based tissue cultures. The carrier plates are designed in a 24-well or in a modified 6-well shape according to requirements of ANSI/SLAS standards 1 and 4 (Figures a and b). The corresponding BRAND*plates*® Insert Strips consist of 4 inserts in a row (Figure c) and are hold in a fixed position at any time of automated handling.

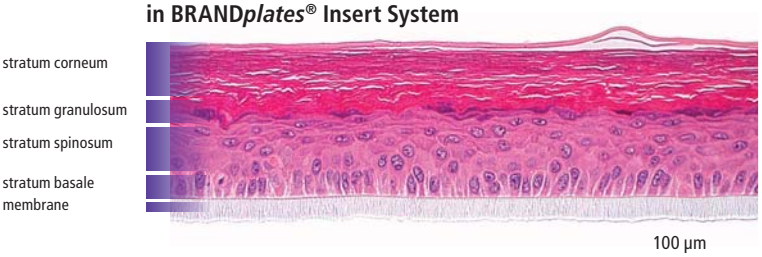
6-well plate
- Use just one or two inserts per well to extend medium change interval
- For up to 4 inserts, medium in the well can be changed in one step

Inlet Opening System (IOS)
- No leaking during cell seeding or initial coating
- Simultaneous change of medium in the well and insert
- Set-up of air-liquid interface in one step
- Compatible with 24- and 6-well BRAND*plates*®



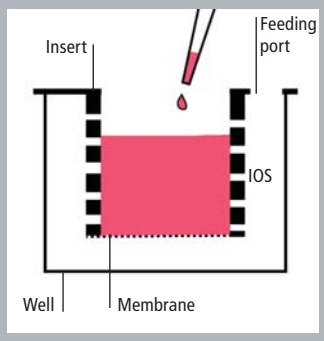


Representative reconstructed human epidermis cultivated in BRAND*plates*® Insert System

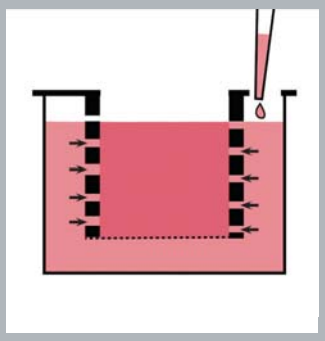


(picture © Fraunhofer/Rafael Krötz)

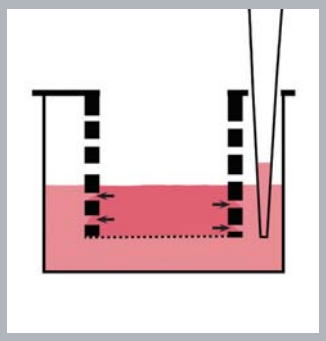
BRAND®



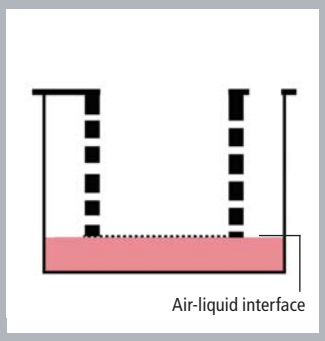
1) Coating, cell seeding, IOS (Inlet Opening System)



2) Medium application for submerge culture



3) Medium change



4) Establish air-liquid interface

BRAND*plates*® Inserts are available with the Inlet Opening System (patent pending, Figure c) which is dedicated to support the automated in vitro reconstruction of human skin. This peerless feature interconnects the medium of wells and inserts enabling the establishment of an air-liquid interface without entering the inserts with pipette tips. In addition to this increase in safety for cultures, the IOS reduces the number of pipetting steps needed to change medium within the two compartments.

Detailed instructions for reconstructing human epidermis and full skin models at www.brand.de

BRAND*plates*® Insert Strips

Insert strips, smooth-walled or with inlet channels (Inlet Opening System*)
PS cellGrade™ plus surface, sterile. Strips of 4 inserts (divisible). Individually wrapped.

Description	Pore size (µm)	Pk	PC membrane Cat. No.	PET membrane Cat. No.
Smooth walled	0.4	12	732-1651	732-1659
With Inlet Opening System	0.4	12	732-1655	732-1663

**Patent pending.*

BRAND*plates*® Insert System

6-well plates filled with 6 insert strips
PS cellGrade™ plus surface, sterile. Insert strips, smooth walled or with inlet channels (Inlet Opening System*). Inclusive lid with condensation rings.

Description	Pore size (µm)	Pk	PC membrane Cat. No.	PET membrane Cat. No.
Smooth walled	0.4	5 (30 insert strips)	732-1676	732-1678
With Inlet Opening System	0.4	5 (30 insert strips)	732-1677	732-1679

**Patent pending.*

BRAND*plates*® plates

Standard plates or 6-well plates
PS pureGrade™ S or cellGrade™ plus surface, sterile. Inclusive lid with condensation rings. Individually wrapped.

Description	Surface	Pk	Cat. No.
24-well standard plate	pureGrade™ S	10	732-1684
6-well plate	pureGrade™ S	10	732-1650
24-well standard plate	cellGrade™ plus	10	732-1685
6-well plate	cellGrade™ plus	10	732-1649

**Patent pending.*

The Nunclon Sphera surface supports formation of three dimensional cancer spheroids in suspension

Louise Gaarn, Tina Marwood, Robert Scott,
Stephanie Carter, Joseph Granchelli, and Cindy Neeley

KEY WORDS

Nunclon Sphera, cancer spheroid, low cell binding, 3D cell culture

ABSTRACT

While monolayer cell culture has been used extensively to study many types of cancers ***in vitro***, 3 dimensional (3D) cultures of cancer spheroids better simulate the in ***vivo*** environment and, therefore lead to a better understanding of tumor biology. Here we introduce a new cell culture surface, Thermo Scientific™ Nunclon™ Sphera, that supports the ***in vitro*** formation of cancer spheroids consistently. The Nunclon Sphera surface coating inhibits cell attachment to the culture dish by blocking the adsorption of extracellular matrix (ECM) proteins (e.g. Collagen I and Fibronectin) that usually mediate cell adhesion. This was further supported by results in low cell attachment of adherent cell lines (e.g. Vero, A549, and U937) to the Nunclon Sphera surface. The functionality of the surface was demonstrated by the formation of cancer spheroids of several commonly used cancer cell lines including HeLa, MCF-7, HepG2, Panc-1, Saos-2, and A549 on the Nunclon Sphera dishes. The integrity and consistency of the Nunclon Sphera surface was showcased by spheroid culture of a cancer stem cell line, P19.CL6. When seeded at relatively low cell seeding densities, P19.CL6 spheroids were able to grow in volume over time. The performance of Nunclon Sphera in promoting cancer spheroid formation was comparable to that of a similar product by a different manufacturer, whereas use of a non-treated Petri dish resulted in significant cell attachment and failed to support cancer spheroid formation.

INTRODUCTION

The study of cell biology *in vitro* via monolayer cell culture systems is not always an accurate representation of the complex environment these cells can experience *in vivo*. Significant interaction that occurs between cells and with the extracellular matrix (ECM) is often not reflected in these simplified culture systems. Three dimensional (3D) cell culture systems better mimic complex interactions and are extremely useful in broad applications of cell biology. In human cancer biology, a 3D culture system can be used to form spheroid cell aggregates that simulate the 3D structure of tumor growth for the purpose of studying tumor cell progression and sensitivity to anticancer agents. However, variability in forming cancer spheroids has been a persistent problem that has been linked to changes in medium composition, volume, cell density, duration in culture, and most importantly the cellular interactions with the culture dish itself. More consistent results can be achieved using a high quality culture surface with very low binding characteristics. In this application note, we introduce a hydrophilic polymer coated surface, Thermo Scientific Nunclon Sphera, which minimizes surface variability and supports the formation of consistent cancer spheroids *in vitro*.

MATERIALS AND METHODS

ECM Protein Non-specific Binding

Nunclon Sphera 96-well plates and Nunclon Delta (standard cell culture-treated) 96-well plates were coated with 100 µL/well of either 24 µg/mL FITC labeled Bovine Collagen Type I or 20 µg/mL of TAMRA labeled Fibronectin in DPBS. The plates were incubated for 24 hours at 2-8°C or 16 hours at room temperature, respectively. The solution was aspirated and plates were washed 3 times with 200 µL/well PBST (0.05% Tween 20 in PBS). The fluorescence intensity was read at Ex495/Em525 (Collagen) or Ex543/Em570 (Fibronectin) on a POWERSCAN MX (DS pharma).

Cell Adhesion

U937 cells underwent a differentiation step prior to adhesion studies. Briefly, U937 cells were cultured for 1 day in media containing phorbol 12-myristate 13-acetate (PMA) at concentration 10 ng/mL. Cells were then cultured for 2 more

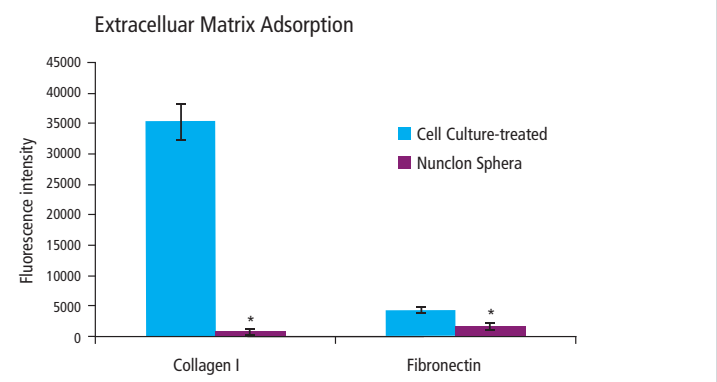


Figure 1. The adsorption of Collagen I and Fibronectin to the Nunclon Sphera surface is extremely low compared to the standard cell culture-treated surface (*, Student's T test, p<0.01).

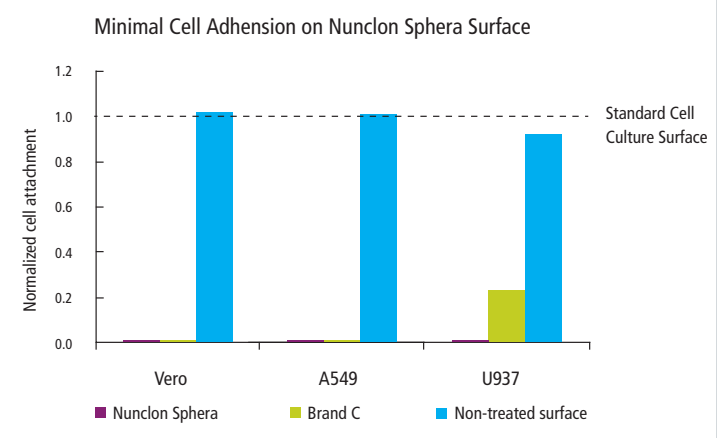


Figure 2. Cell adhesion is much lower in the Nunclon Sphera surface than in cell culture-treated and non-treated controls for all cell types tested. Nunclon Sphera also shows significantly lower adhesion than the Brand C surface with U937 cells.

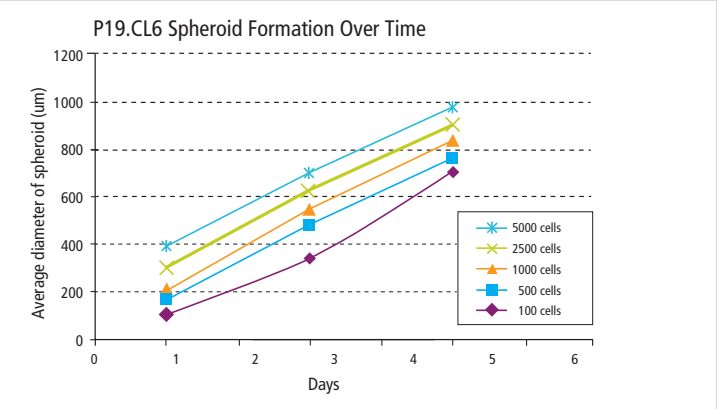


Figure 3. Cancer stem cell spheroids grow in size over time (average of 4 separate measurements) when cultured on the Nunclon Sphera surface at a rate associated with the initial seeding density.

days in fresh media without PMA.
A similar protocol was used for cell adhesion assays for the differentiated U937 cell line and 2 other cell lines (VERO and A549). Briefly, cells were seeded onto 6-well multidishes at $1.5x10^5$ cells/cm² (U937) or $4x10^4$ cells/cm² (VERO and A549). After a 30 minute (U937) or 24 hour (VERO, A549) incubation at 37°C and 5% CO_2, culture dishes were washed and residual cells lysed with 2% Triton X-100 in phosphate buffered saline (DPBS). Dishes were incubated for another 30 minutes at 37°C before 100 µL of lysate was transferred to each well of a 96-well plate. An assay was performed using a commercial kit (Roche, 11644793001) to obtain the fluorescent intensity of the samples at 490 nm wavelength. Sample readings were compared to standards made with known quantity of cells to determine the amount of cell adhesion in the culture dishes. Results were normalized to the standard cell culture treated culture dishes.

Cancer Cell Line Cultivation

Cancer cell lines (HeLa, HepG2, A549, MCF-7, P19.CL6, Panc-1, and Saos-2) were maintained on the Nunclon Delta surface in the appropriate HyClone media containing 10-15% FBS (HyClone, SH3007103) at 37°C and 5% CO_2 before they were subjected to cancer spheroid formation in various low cell binding dishes.

Spheroid Formation

Cell lines HeLa, HepG2, A549, and MCF-7 were seeded into Nunclon Sphera and Brand C 6-well multidishes at a density of $2x10^4$ cells/well (HeLa, HepG2, A549) or $4x10^4$ cells/well (MCF-7) in the appropriate HyClone media containing 10% FBS (Hyclone, SH3007103). Untreated and cell culture-treated dishes were also seeded as controls. Cells were incubated at 37°C and 5% CO_2, and fresh media was added after 2-3 days. After 7 days cells were imaged, the size of spheres was measured, and the number of spheres and total cell count per well were determined.
To monitor the effect of the Nunclon Sphera coating on cancer spheroid growth, the P19.CL6 cancer stem cells were plated at density of 100, 500, 1,000, 2,500 and 5,000 cells/well in Nunclon Sphera 96 well U bottom plates. Cells were incubated for 5 days and spheroid size was measured by microscopic examination on days 1, 3, and 5.

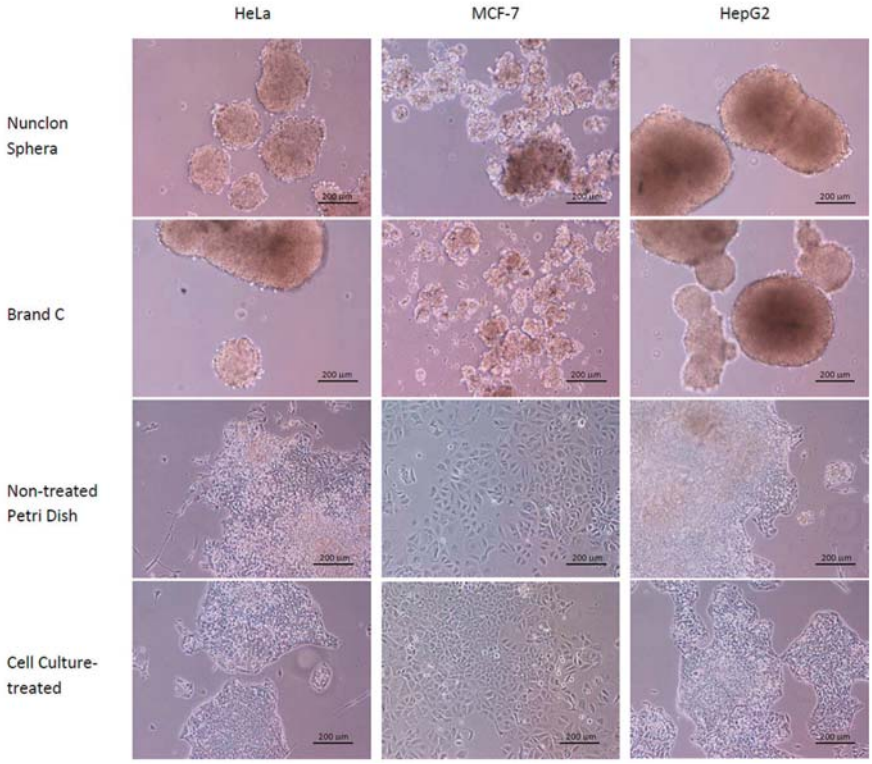


Figure 4. Nunclon Sphera effectively supports the formation of sizable cancer spheroids in culture. HeLa, MCF-7, and HepG2 cells were grown in Nunclon Sphera, Brand C, non-treated Petri, and standard cell culture-treated dishes.

RESULTS AND DISCUSSION

The adsorption of ECM to the Nunclon Sphera surface is extremely low

Common ECM proteins are known to mediate cell attachment to culture surfaces. In order for adherent cells to form spheroids in suspension, the culture vessel must encourage the aggregation of cells through cell-cell binding by preventing ECM binding to the plastic surface. In this study, both Collagen I and Fibronectin binding was minimal on the Nunclon Sphera surface. This is demonstrated through the extremely low fluorescence intensity following overnight incubation of solution with the ECM proteins (Figure 1). This suggests that unlike the standard cell culture-treated surface, the Nunclon Sphera surface has minimal binding interactions with the ECM, consequently discouraging the cells from attaching to the surface.

Surface adhesion of cells is extremely low on Nunclon Sphera

In order to verify that adherent cells show little attachment to the Nunclon Sphera surface, the quantity of adhered cells was assayed in cell culture dishes with either the Nunclon Sphera surface, a low cell binding surface by a different manufacturer (Brand C), or the non-treated polystyrene surface commonly used for non-adherent cell culture. A standard cell culture-treated surface was used as a positive control for cell adhesion. The Nunclon Sphera surface demonstrated virtually no cell adhesion for all cell lines tested whereas the Brand C attracted some differentiated U937 cells to the surface (Figure 2).

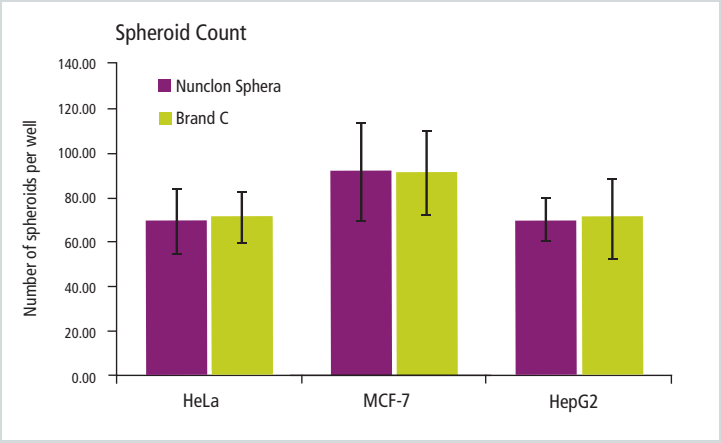
The non-treated polystyrene surface showed significant cell adhesion, comparable to the cell culture-treated surface, presumably due to the presence of the serum and abundant ECM proteins in the culture system (Figure 2). While the non-treated polystyrene surface is unsuitable for spheroid culture, the Nunclon Sphera surface with the low adhesion properties demonstrates its feasibility in applications where preventing cells from binding to the culture dish are desired.

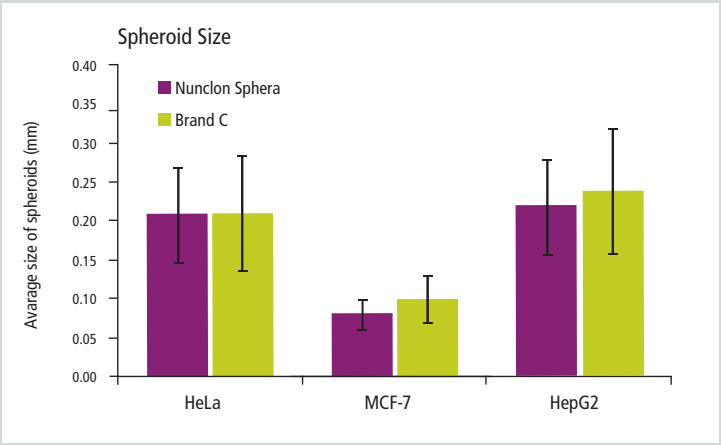
The Nunclon Sphera surface has no deleterious effects on cell growth

While cells do not adhere to the Nunclon Sphera, it is also critical to verify that the surface coating does not hamper the normal growth of the culture. To demonstrate cell growth, P19.CL6 mouse cancer stem cells were plated at 5 different densities on the Nunclon Sphera surface and spheroids were able to grow in size for 5 days. There was an approximate 6.5 fold increase in spheroid size at the lowest seeding density while there was only a 2.5 fold increase at the highest seeding density (Figure 3). These results suggest that the seeding density affects the growth rate of spheroids. Additionally, the spheroids of all initial seeding densities were able to grow in volume, indicating that the Nunclon Sphera polymer coating has no adverse effect on cell survival and proliferation.

The Nunclon Sphera surface supports spheroid formation and growth of multiple cancer cell lines

The key element for any low cell binding surface is its performance in forming and growing 3D spheroids in culture. To test this, several cancer cell lines were grown in dishes of various low cell binding surfaces, including the Nunclon Sphera surface, the Brand C surface by a different manufacturer, and the non-treated Petri dish. The standard cell culture-treated Nunclon Delta dish was used as a negative control for spheroid formation. The formation and growth of spheroids were assessed after a week of incubation. Microscopic examination demonstrated that the Nunclon Sphera surface consistently supported spheroid formation of all cancer cells, and the spheroids maintained healthy and normal morphology in culture (Figure 4). Brand C performed comparably to the Nunclon Sphera surface with the spheroids slightly less uniformed in size (Figure 4).





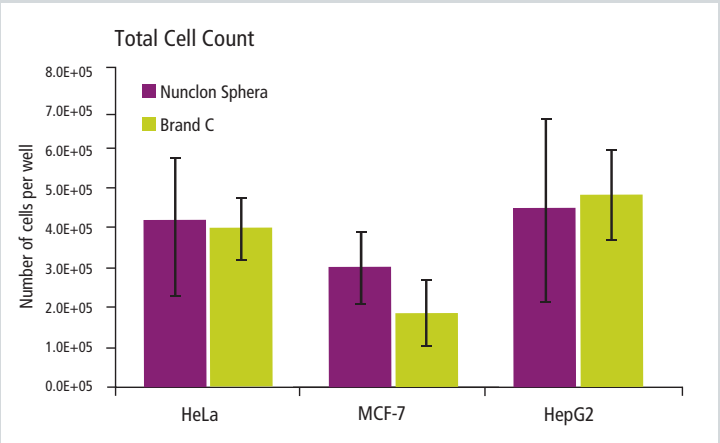


Figure 5. Nunclon Sphera performs as well as the Brand C in the number of spheroids formed, the size of spheroids grown, and the total number of cells grown per well.

Interestingly, the non-treated Petri dish, which is made of hydrophobic polystyrene and is routinely used for suspension cell culture, failed to support cancer spheroid growth resulting in significant cell attachment of all cancer cell lines tested (Figure 4). In addition to the HeLa, MCF-7, and HepG2 cells shown, similar results were obtained with Panc-1, Saos-2, and A549 cells (data not shown).

To quantify the performance of the surfaces, assessments were made for the number and size of the cancer spheroids, as well as the total number of cells in the well after a week of incubation. The Nunclon Sphera surface achieved similar results as the Brand C surface in all categories, with no significant differences in the number of spheroids, the size of the spheroids, or the total number of cells (Figure 5).

CONCLUSIONS

The Nunclon Sphera surface consistently demonstrates minimal ECM protein binding, extremely low cell attachment, and supports good formation and proliferation of spheroids across several commonly used cancer cell lines. These results indicate that the Nunclon Sphera surface is an excellent choice for 3D cancer spheroid culture.

- Minimal adsorption of ECM proteins and extremely low cell adherence highlight the low binding properties of the Nunclon Sphera surface.
- The Nunclon Sphera polymer coating has no adverse effects on cell survival and proliferation as demonstrated by cancer spheroids with low seeding density grown both in number and size over time.
- The Nunclon Sphera surface effectively supports the formation of spheroids by many different cancer cell lines, providing a consistent culture system for spheroid growth and aiding in 3D cancer cell modeling *in vitro*.

Description	Units per bag	Units per case	Cat. No.
Microwell 96U-Well Plate, Round Bottom, Well Volume 300 µL	1	8	392-0607
Microwell 96F-Well Plate, Flat Bottom, Well Volume 400 µL	1	8	392-0608
Multidish 24-Well, Culture Area 1.9 cm^2	1	7	392-0609
Multidish 12-Well, Culture Area 3.8 cm^2	1	7	392-0610
Multidish 6-Well, Culture Area 9.6 cm^2	1	7	392-0611
Dish 35 MM, Culture Area 8.8 cm^2	5	20	392-0612
Dish 60 MM, Culture Area 21.5 cm^2	5	20	392-0613
Dish 90 MM, Culture Area 56.7 cm^2	5	20	392-0614
T25 Cell Culture Flask, Culture Area 25 cm^2	6	18	392-0615
T75 Cell Culture Flask, Culture Area 75 cm^2	4	24	392-0616

The RNAi-cap™ Technology increases siRNA potency and decreases off-target effects

Riboxx GmbH, Meissner Straße 191, D-01445, Germany
info@riboxx.com

Gene silencing using siRNA is a well established method for investigation of genotypic/ phenotypic correlations in life sciences, in particular in the field of cancer research and degenerative diseases. The majority of the commercially available siRNA display the so-called "Tuschl design", with overhangs located at the 3´-End of the siRNA. A major challenge of this design relates to increasing potency of gene silencing and reducing off-target effects. Suppliers address these issues by improving the algorithm used to design siRNA.
Riboxx has invented a new technology termed the RNAi-cap™ that increases the potency of siRNA and reduces off-target effects independently from the algorithm used.
In this study, the RNAi-cap™ Technology has been applied to various siRNA from three different suppliers [A; B, and C]. The potency of gene silencing between the standards siRNA with 3´-overhangs and the siRNA displaying the RNAi-cap™ technology is compared.

MATERIAL AND METHODS

Cells

MIN-6 cells and MiaPaCa2 cells were purchased from ATCC and cultivated according to manufacturer´s expression. Human Primary endothelial cells were cultivated as previously described (Nolte et al., 2013). For transfection of siRNA, riboxx®FECT transfection reagent was used according to manufacturer´s instructions (Riboxx).

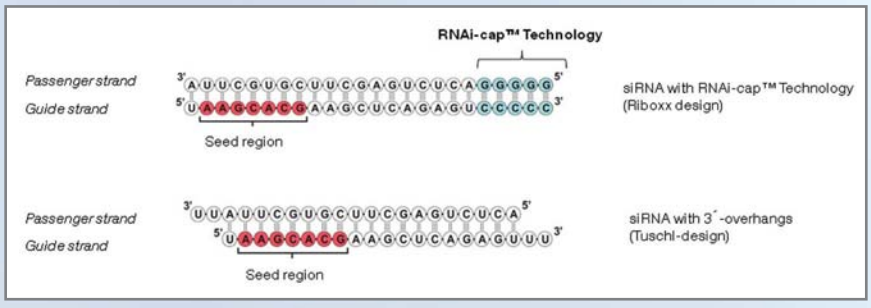


Figure 1. Design of the siRNA with the RNAi-cap™. The seed region is shown, as well as the region bearing the RNAi-cap™. The siRNA has a total length of 24 bp including the RNAi-cap™.

siRNA	Target gene	cells
ICA512siRNA #1	ICA512	MIN-6
ICA512siRNA #2	ICA512	MIN-6
Cav-1 siRNA	Caveolin	MiaPaCa2
Off-targeting siRNA (TNFR-1)	ICAM-1 (Passengerstrand: TNFR-1)	HUVEC
Off-targeting siRNA (ICAM-1)	TNFR-1 (Passengerstrand: ICAM-1)	HUVEC
Non-targeting siRNA	none	MIN-6, MiaPaCa2, HUVEC

Table 1. Characteristics of the siRNA used in this study.

Analysis of gene knock down

Analysis of gene knockdown was performed by Western blot. Housekeeping genes were used as control of protein level. As a negative control for gene silencing, non targeting siRNA was used (iBONi® siRNA negative control, Riboxx). Alternatively, qRT-PCR was performed for determination of gene knock down.

RESULTS AND DISCUSSION

The RNAi-cap™ Technology can be applied to any siRNA

Six different siRNA's targeting different genes and different cell types were designed using different algorithms from commercial suppliers. The siRNAs are displayed in Table 1.

As shown in Figure 1, the RNAi-cap™ is located on the opposite side of the seed region of the siRNA. It can be incorporated to any siRNA designed by any algorithm, independently of the algorithm chosen. The RNAi-cap consists of a stretch of 5Cs annealed to 5Gs.

The RNAi-cap™ Technology strongly increases the potency of the siRNA

Standard siRNA bearing 3´-overhangs were purchased from commercial suppliers, as shown in Table 1. The siRNA targeted the ICA512 or caveolin-1 gene, yielding a moderate knock down of gene expression.
By adding the RNAi-cap™ to the siRNA, knock down potency was increased, as evidenced in Figure 2.

riboxx PHARMACEUTICALS

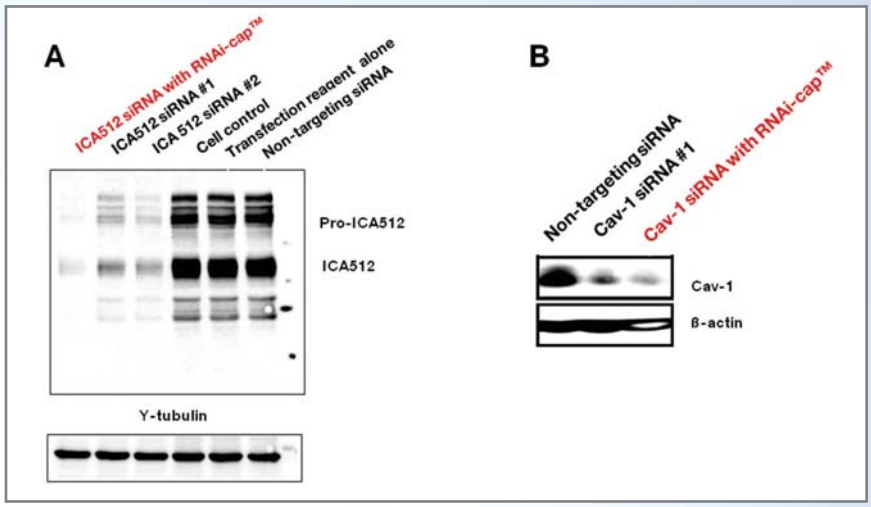


Figure 2. The RNAi-cap™ Technology strongly increases the potency of the siRNA
A, Knock down of ICA512 gene expression in MIN6 cells by siRNA bearing the RNAi-cap™ in comparison to two different siRNAs (ICA512 #1 and ICA512 siRNA #2) with standard 3´-overhangs designed with algorithm of supplier [A]. Expression of the ICA512 protein in MIN-6 cells was analysed by western blot. As a control, the expression of the housekeeping gene γ-tubulin is shown.
B, Knock down of caveolin gene expressionin MIaPaCa2 cells by siRNA bearing the RNAi-cap™ in comparison to the same siRNA with 3´-overhangs (cav-1 siRNA) designed with algorithm of supplier [B]. Expression of the caveolin protein in MiaPaCa2 cells was performed by western blot. As a control, the expression of the housekeeping gene ß-actin is shown.

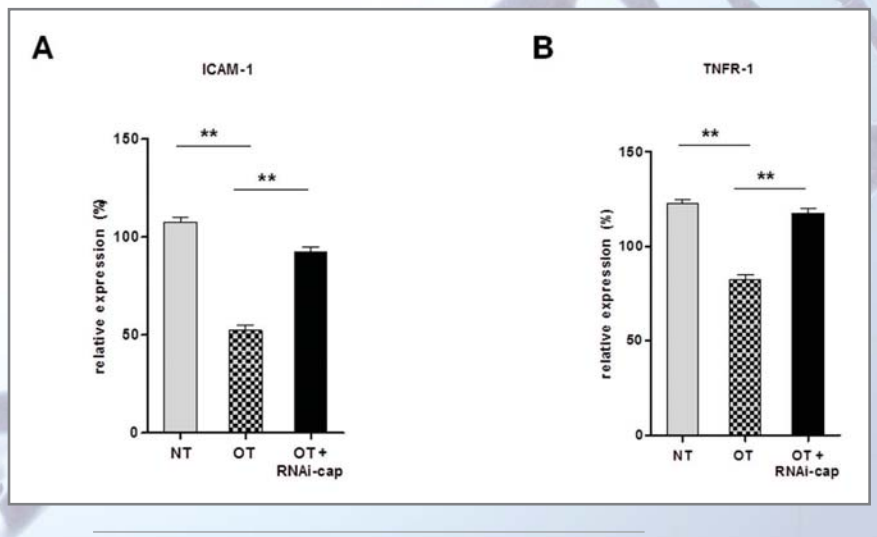


Figure 3. Reduced off-target effects of the siRNA displaying the RNAi-cap™ in comparison to siRNA with 3´-overhangs designed by supplier [C].
A, siRNA off-targeting ICAM-1 (OT) as well as the same siRNA off-targeting ICAM-1 but displaying the RNAi-cap™ (OT+RNAi-cap™) were compared.
B, siRNA off-targeting TNFR-1 (OT) as well as the same siRNA off-targeting TNFR-1 but displaying the RNAi-cap™ (OT+RNAi-cap™) were compared.
As a negative control, a non targeting siRNA (iBONi® siRNA negative control) for both ICAM-1 or TNFR-1 was used. Expression was calculated by setting untransfected, non stimulated cells to 100% expression. Each bar represents the mean ± standard error of mean (SEM) of n = 3. ** indicates statistical significance at a level of P <0.01.

The RNAi-cap™ Technology reduces off-target effects of siRNA

In order to measure the reduction of off-target effects by the RNAi-cap™ Technology, two siRNA off-targeting the ICAM-1 gene or the TNFR-1 gene were used (Table 1). The off-target effects of the siRNAs were compared in the presence or the absence of the RNAi-cap™ Technology. As a control, a non targeting siRNA (iBONi® siRNA negative control) for both ICAM-1 or TNFR-1 was used. Expression of ICAM-1 or TNFR-1 was performed by qRT-PCR as already described (Nolte et al., 2013).

CONCLUSION

The RNAi-cap™ Technology developed at Riboxx increases potency of siRNA and reduces off-target effects. The RNAi-cap™ technology is applicable to any siRNA designed by any algorithm, from any supplier. The RNAi-cap™ Technology is available at Riboxx in different siRNA and microRNA mimics formats (iBONi® siRNA, iVORi® siRNA, CONmiR® miRNA mimics).

References

Nolte A, Ott K, Rohayem J, Walker T, Schlensak C, Wendel HP. Modification of small interfering RNAs to prevent off-target effects by the sense strand. N Biotechnol. 2013 Jan 25;30(2):159-65.

Quanta microRNA Technology

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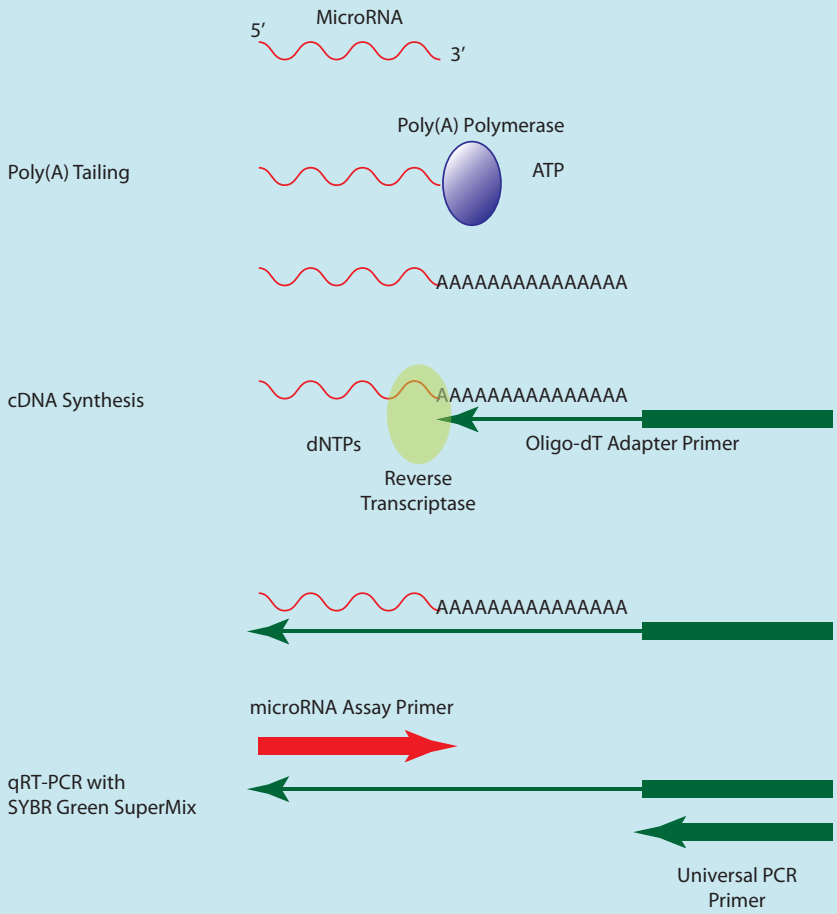
- **Detect microRNAs with log-fold greater sensitivity than currently available systems**
- **Profile microRNAs from small amounts of sample**
- **Integrated system includes positive control and does not require expensive hydrolysis probes**
- **Broad dynamic range of RNA input**



Quanta microRNA Technology
The qScript microRNA cDNA Synthesis Kit is suitable for use with total RNA or preparations pre-enriched for microRNAs. A poly(A) tail is added to microRNAs followed by cDNA synthesis using an adapter primer and qScript RT in a single-tube reaction. The resulting cDNA is ready for qPCR amplification with a Universal PCR Primer and your choice of microRNA Assays. This unique single-tube system provides ease-of-use and efficient cDNA synthesis for quantification of microRNAs with a high degree of sensitivity and specificity. PerfeCTa™ SYBR Green SuperMixes ensure compatibility with any real-time thermal cycler.

Confidence in your results

Quanta's technology allows inclusion of a minus-poly(A) polymerase control not available with other protocols. Each kit also includes a positive control for SNORD44 that may also be used as a calibration target.

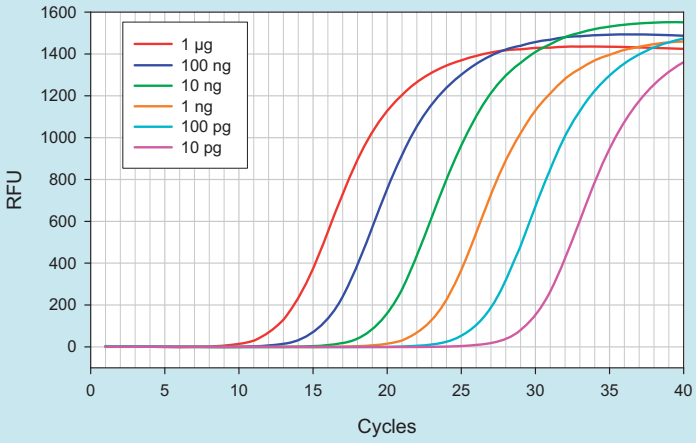
Detection of rare microRNAs

Quanta's microRNA profiling system provides linear detection and quantification of microRNAs across total RNA input levels spanning six orders of magnitude. This means microRNAs will be detected when present even when tissue is scarce or the microRNAs are rare.

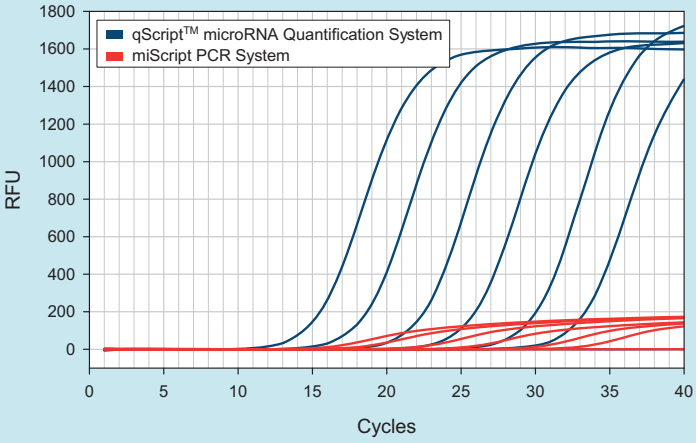
Superior sensitivity

Quanta's microRNA profiling system yields superior results when compared to other leading microRNA quantification systems (Figure 3). The qScript™ microRNA cDNA Synthesis Kit, PerfeCTa microRNA Assays and PerfeCTa SYBR Green SuperMix form an integrated system that yields industry leading results.

Quanta BIOSCIENCES™



Detection of rare microRNAs
Quanta's microRNA profiling system provides linear detection and quantification of microRNAs across total-RNA input levels spanning six orders of magnitude. This means microRNAs will be detected when present even when tissue is scarce or the microRNAs are rare.



qScript™ microRNA Quantification System comparison to Qiagen (hsa-let-7a)
microRNA cDNA synthesis was performed starting with log-fold dilutions of human brain total RNA (from 1.0 µg to 10 pg) using the qScript™ microRNA cDNA Synthesis Kit (Quanta) or the miScript Reverse Transcription Kit (Qiagen). 1/100th of each microRNA cDNA reaction was used in a 20µl qPCR amplicfication reaction specific for hsa-let-7a using each manufacturers validated microRNA assay, qPCR mix and recommended protocol. Averaged plots for triplicate qPCR reactions for each input quantity are shown. Cycling conditions for the miScript Primer Assay with the miScript SYBR Green PCR Kit (Qiagen) consisted of 94°C, 15 min followed by 40 cycles of 94°C, 15s; 55°C, 30s and 70°C, 30s. Cycling conditions for the PerfeCTa® microRNA Assay and PerfeCTa® SYBR® Green SuperMix™ (Quanta) consisted of 95°C, 2 min followed by 40 cycles of 95°C, 5s and 60°C, 30s.

qScript™ microRNA cDNA Synthesis Kit

The qScript™ microRNA cDNA Synthesis Kit has been designed to convert miRNAs into cDNA starting from total RNA or RNA preparations pre-enriched for miRNAs. miRNAs are not polyadenylated in nature. With the qScript™ microRNA cDNA Synthesis Kit miRNAs are polyadenylated in a poly(A) polymerase reaction. qScript™ Reverse Transcriptase and other necessary reagents for cDNA synthesis are subsequently added to convert the poly(A) tailed microRNAs into cDNA using an oligo-dT adapter primer. The adapter primer has a unique sequence at its 5? end which allows amplification of cDNAs in Real-Time qRT-PCR reactions.

The kit comes complete with a Human Positive Control Primer that can be used to quantitate the small nucleolar RNA SNORD44 which is ubiquitously expressed in most human tissues. In addition, the kit contains 20% extra Poly(A) Tailing Buffer and MicroRNA cDNA Reaction Mix to accommodate the use of no poly(A) polymerase and no reverse transciptase control reactions.

Individual microRNAs are quantitated in Real-Time qRT-PCR amplification reactions using the PerfeCTa microRNA Assays along with the PerfeCTa Universal PCR Primer (specific to the unique sequence of the oligo-dT adapter primer) and the PerfeCTa SYBR Green SuperMix family of products. The pre-designed and validated PerfeCTa MicroRNA Assays provide maximum sensitivity and specificity in qRT-PCR amplifications. For a complete list of available microRNA assays please visit www.Quantabio.com/microRNA.

qScript™ microRNA cDNA synthesis kit	Pk	Cat. No.
25×20 µl reactions	25 assays	733-2013
100×20 µl reactions	100 assays	733-2014

5 PRIME

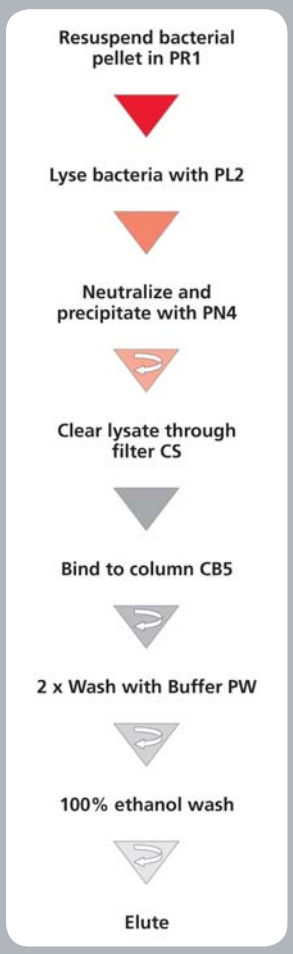
PerfectPrep EndoFree Plasmid Maxi Technology

Purification of endo-free, transfection grade plasmid DNA on a large scale

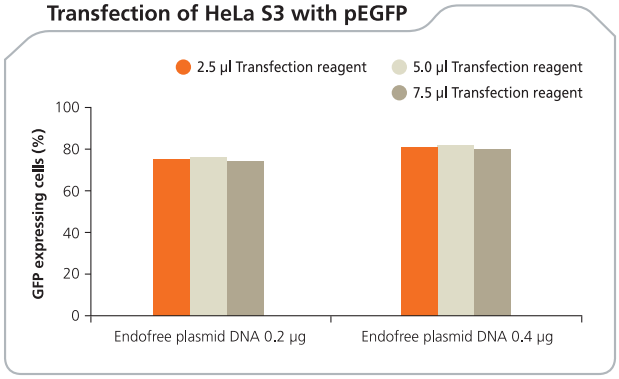
FEATURES AND BENEFITS

- Rapid and simple purification – in less than 40 minutes
- Up to 1000 µg of high quality plasmid DNA, e.g. transfection
- High mammalian cell viability post-transfection due to efficient endotoxin removal

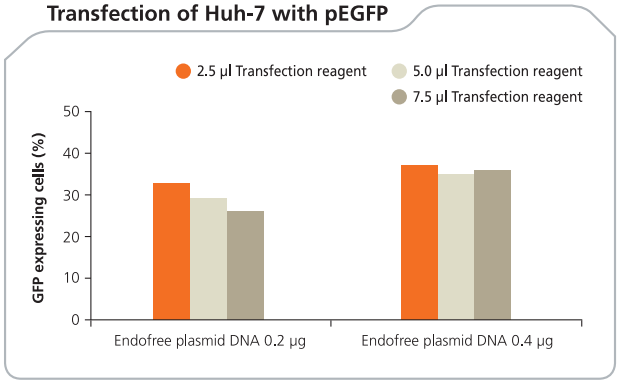
PerfectPrep Endofree Maxi Procedure



The PerfectPrep EndoFree Maxi Kit generates high quality plasmid DNA with an endotoxin removal process, for use in advanced downstream applications. The efficient endotoxin removal (<0.1 endotoxin units per µg plasmid DNA) results in improved mammalian cell viability. This kit is designed to isolate plasmid DNA of the highest purity from 100 ml of bacterial cultures for high copy plasmids or 250 ml of bacterial culture for low copy plasmid yielding up to 1000 µg of transfection grade plasmid DNA. The PerfectPrep EndoFree Maxi Kit includes PrefectPrep EndoFree Filter CS, which allows the clarification of the bacterial lysate. Following this the DNA is purified with a silica membrane. The resulting plasmid DNA is highly suited for use in a broad variety of demanding applications, including transfection of sensitive and primary cell lines, in vitro transcription and translation and all enzymatic modifications.



Excellent transfection results with endotoxin-free plasmid DNA using different amounts of transfection reagent.



Maximum recommended culture volume and yields			
Plasmid type	**Yield**	**Culture volume***	**Binding capacity of the column**
High copy	500 to 1000 µg	100 ml	1 mg
Low copy	300 to 400 µg	250 ml	1 mg

**Actual yields depend on plasmid copy number, size of insert, host strain, culture medium and culture volume, especially the state of the bacterial.*

Endotoxin levels and transfection efficiency according to method

The transfection efficiency particularly for sensitive and primary cell lines depends on the endotoxin level of the plasmids. The following table summarises typical results using different purification methods.

Plasmid preparation method	Endotoxin (EU†/µg DNA)	Average transfection efficiency‡
PerfectPrep EndoFree Plasmid Kit	0.1	154%
5 PRIME Plasmid Kits	9.3	100%
2x CsCl	2.6	99%
Silica slurry	1230.0	24%

** Host strain: DH5a; plasmid: pRSVcat.*
† 1 ng LPS = 1.8 EU.
‡ The transfection efficiency obtained using plasmid prepared with 5 PRIME Plasmid Kits was set to 100%. The transfection efficiencies for all other preparation methods were calculated relative to the 5 PRIME Plasmid Kit.

Description	Pk	Cat. No.
PerfectPrep EndoFree Maxi Kit	10 preps	733-1942

VWR® mySpec

VWR introduces the new mySPEC series of spectrophotometers:

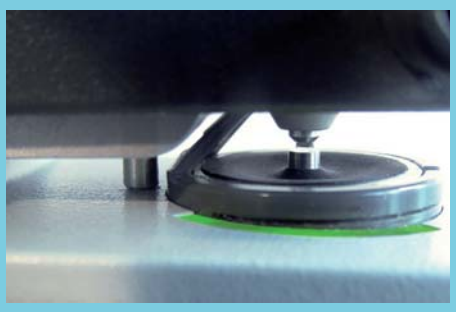
Cutting edge technology for the next generation in microvolume measurement instrumentation

The VWR mySPEC microvolume spectrophotometers combine gold standard detection technology with new levels of user interaction and data analysis.
The VWR mySPEC models enable highly accurate UV/Vis analyses of 1 µl samples with remarkable reproducibility. Processing of samples is more robust than ever before.

The sophisticated sample retention system of all VWR mySPEC instruments eliminates the need for cuvettes, capillaries or other consumables resulting in decreased measurement cycle times and full cost control.

Available with microvolume sample retention system only or additional cuvette capabilities controlled either by an external laptop or onboard touch screen PC, the VWR mySPEC is the new system of choice for the busy and modern life science laboratory.

- **Determination of nucleic acid concentrations and purities in 1 µl samples**
- **Large dynamic range from 2 ng/µl to 15 µg/µl (ds DNA)**
- **Complete spectral range (190 – 840 nm)**
- **Protein measurements (A280, Bradford, Lowry, BCA, Pierce 660)**
- **Determination of labelling efficiencies, e.g. for microarray experiments**
- **Colorimetric assays on enzymatic activities and cell density measurements**
- **'Twin' versions combining microvolume and cuvette function including temperature control (30 – 40 °C) and stirrer**



Pipette, measure and wipe: Obtain full spectral data within 5 seconds.

Description		Cat. No.
VWR mySPEC	For microvolume samples and external computer control	732-2533
VWR mySPEC Twin	For microvolume + cuvette measurements and external computer control	732-2534
VWR mySPEC Touch	For microvolume meassurements; standalone system	732-2535
VWR mySPEC Twin Touch	For microvolume + cuvette meassurements; standalone system	732-2536

Not available in Germany and Austria

VWR® TEMPase Hot Start Polymerase

VWR TEMPase Hot Start DNA Polymerase is a chemically modified form of Taq Polymerase. A chemical moiety is attached to the enzyme, which reversibly inactivates the TEMPase. This allows convenient reaction set-up at room temperature and even plate preparation up to 2 days in advance. TEMPase is activated by heat treatment during the first heating step.

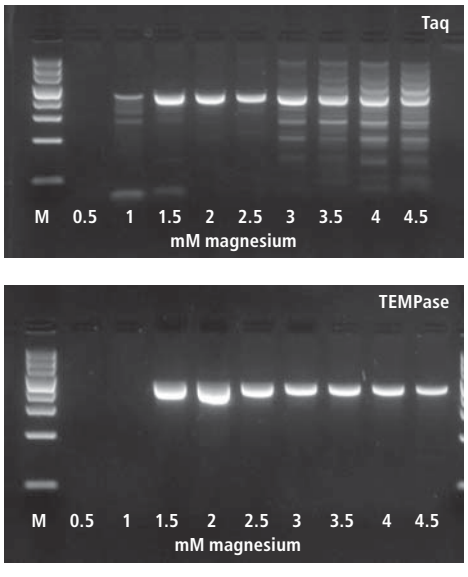


Figure 1. Superior specificity and high yields. BAIP3 gene product was amplified in a PCR using Taq Polymerase or TEMPase Hot Start DNA Polymerase and Key Buffer with a magnesium dilution series from 0.5 to 4.5 mM in 0.5 mM increments. Taq Polymerase results in good yield and satisfactory specificity (lanes 1.5 to 2.5). With TEMPase Polymerase, an increase in yield (lanes 1. 5 to 2.5) and specificity (lanes 1.5 to 4.5) is seen. M: Marker.

HIGH YIELDS, SUPERIOR SPECIFICITY AND EXCELLENT SENSITIVITY

The complete inactivity at room temperature and during the first ramp of thermal cycling leads to decreased formation of non specific priming and prevents false amplification. This results in higher specificity, increased sensitivity and greater yields when compared to standard DNA polymerases (Figures 1 and 2).

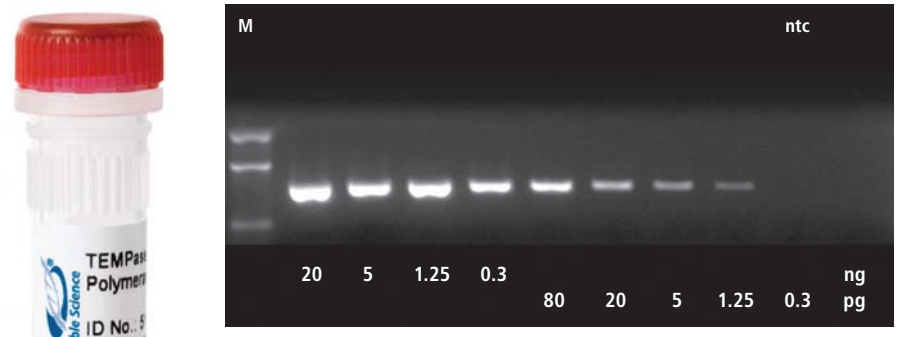


Figure 2. Excellent sensitivity. TEMPase Hot Start Polymerase has high sensitivity and is able to detect as little as 1 copy of a gene. In this experiment the indicated amounts of DNA were amplified in a PCR using TEMPase and Key Buffer. DNA quantities are given in ng or pg under each lane. M: marker; ntc: no template control.

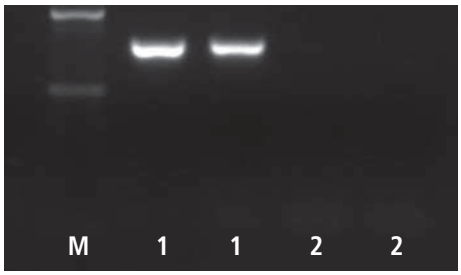


Figure 3. TEMPase is inactive at ambient temperature.
VWR TEMPase is activated by initial heating at 95 °C for 15 minutes (lane 1). Without activation, the enzyme is completely inactive (lane 2). M: marker.

ADVANTAGE OF CHEMICAL INACTIVATION

Chemical inactivation of TEMPase is highly effective (Figure 3). Therefore, the modified enzyme withstands longer periods of time at room temperature without any non specific PCR amplification compared to, for example, antibody inactivated enzymes. This feature is also useful when pre-incubation steps at elevated temperatures are required, such as UNG treatment at 50 °C prior to PCR.

Blue TEMPase Master Mix – for direct gel loading

VWR Blue TEMPase Master Mix is a convenient alternative to TEMPase Hot Start DNA Polymerase and TEMPase Hot Start Master Mix with the same excellent performance. The blue loading dye and stabiliser present in Blue TEMPase Master Mix do not interfere with the PCR reaction. These components facilitate direct gel loading and eliminate the necessity for separate loading dye (Figure 4). There is no need for time-consuming sample preparation before electrophoresis.

Figure 4. Direct gel loading.
After PCR using Blue TEMPase Master Mix, the products are loaded directly on the agarose gel. Lanes 1 and 2: two different primer sets; lanes N: no template control; lane M: marker.

GC-RICH DNA AMPLIFICATION

TEMPase Hot Start DNA Polymerase is well suited for amplification of GC-rich DNA targets. Combined with GC Buffer I and GC Buffer II, TEMPase Hot Start DNA Polymerase promotes excellent amplification results with targets of varying degrees of GC content (Figure 5). The thorough heat activation step required for TEMPase is beneficial when amplifying GC-rich DNA sequences.

MULTIPLEX PCR

Multiplex PCR is a method to amplify several products from the same DNA sample in one tube. TEMPase Hot Start DNA Polymerase is well suited for multiplex PCR due to its high specificity (Figure 6).

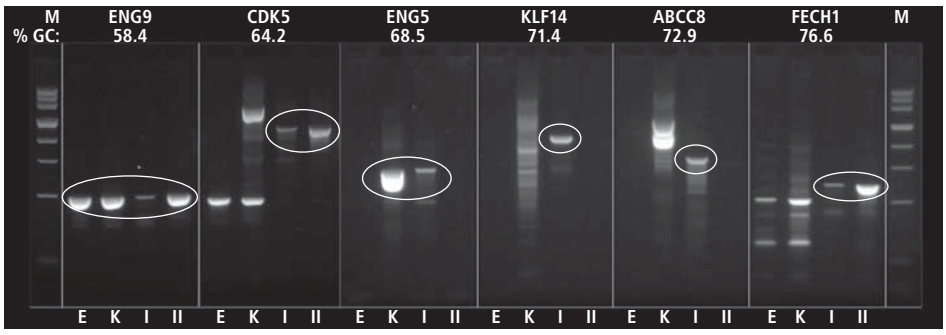


Figure 5. Amplification of GC-rich DNA sequences with diverse buffers.
The indicated six genes with a varying percentage of GC content were amplified with Extra Buffer (lanes E), Key Buffer (lanes K), GC Buffer I (lanes I) and GC buffer II (lanes II). With an increasing percentage of GC in the expected amplicon, Extra Buffer and Key Buffer fail to give the correct amplification products while GC Buffer I or GC Buffer II succeeds. M: marker. Notice: Key Buffer is the buffer of choice for most PCR applications. To obtain a good result for ENG5 it is sufficient only to change the buffer from Extra Buffer to Key Buffer.

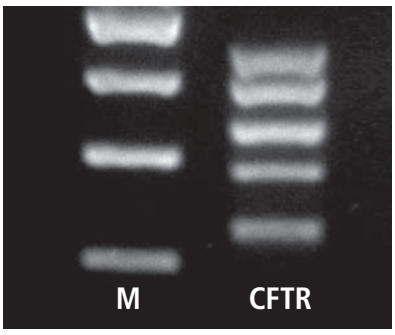


Figure 6. TEMPase performs well in Multiplex PCR.
Five different products of the CFTR gene were simultaneously amplified in one tube using VWR Multiplex TEMPase Master Mix. M: marker; CFTR: the five products of the multiplex reaction.

Unique enzymes from the Arctic alleviate the need to purify samples for several applications

Cloning, protein purification and PCR clean-up all have something in common at AMRESCO – unique recombinant enzymes derived from various arctic species. These enzymes are adapted to cold temperatures and are well suited for standard life science applications, with the added convenience and efficiency of irreversible heat inactivation. Unlike their standard counterpart enzymes, once AMRESCO's cold-adapted enzymes perform their task, their complete inactivation alleviates any requirement for a downstream sample purification step.

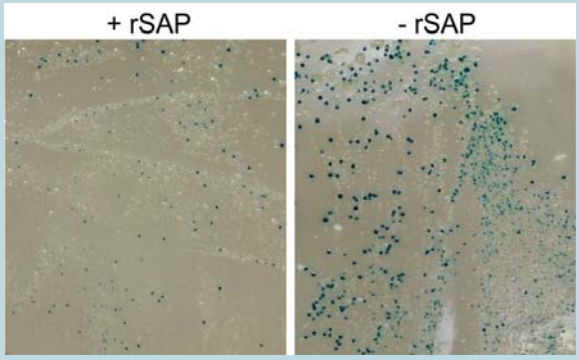


Figure 1A. Shrimp Alkaline Phosphatase, Recombinant (rSAP) treatment reduces empty vector background in molecular cloning. Linearised pUC19 was treated with rSAP or water prior to ligation with an insert. The rSAP was irreversibly heat inactivated and the samples were used without any purification steps before the ligation reaction.

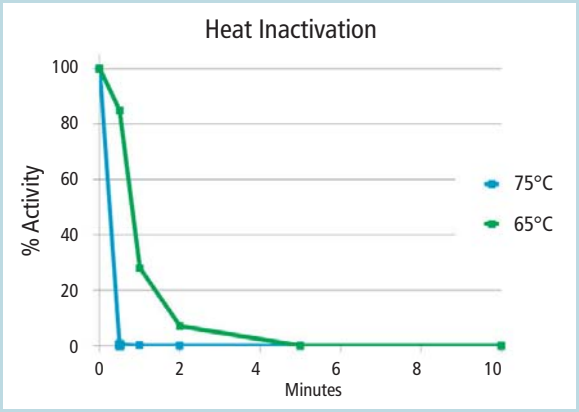


Figure 1B.

Shrimp Alkaline Phosphatase, Recombinant (rSAP)

AMRESCO offers Shrimp Alkaline Phosphatase, Recombinant (rSAP), which non specifically dephosphorylates the 5′ ends of nucleic acids. This activity streamlines molecular cloning by preventing self-ligation of linearised plasmid DNA, thus ensuring low vector background for colony selection. Plasmid DNA may be treated with rSAP concurrently with restriction digestion or by a short, 10 minute incubation post digestion. The rSAP is then irreversibly inactivated by heating to 65 °C for 5 minutes, after which the linearised plasmid may be used directly in a ligation reaction. Other useful applications for rSAP include 5′ end-labelling, for which it facilitates the replacement of unlabelled phosphates with labelled phosphate groups and PCR clean up prior to DNA sequencing or SNP analysis. The latter application is performed by combining the nucleotide dephosphorylating activity of rSAP with the primer degrading activity of Exonuclease I. This effectively removes the ability of unincorporated nucleotides and primers from a PCR reaction to interfere with the primer extension reaction for sequencing or genotyping.

To demonstrate the effectiveness of rSAP activity in molecular cloning, pUC19 digested with SmaI was treated with rSAP or water and then ligated with a 1 kb insert. The ligation products were transformed into competent E. coli and plated in triplicate on LB medium containing ampicillin and AMRESCO's X-Gal/IPTG Ready Solution for blue-white colony screening. Blue and white colonies were compared among the plates for each condition. The number of blue colonies, which represent empty vector, was significantly lower for the plates transformed with rSAP-treated samples than for those that were untreated (Figure 1A). Plasmid isolated from several white colonies on each plate was confirmed by restriction analysis to contain the expected insert (data not shown). These data indicate that rSAP treatment effectively decreased the number of self-ligating vectors prior to ligation with insert, and that rSAP was completely inactivated by heat treatment, allowing ligation between vector and insert with intact 5′ phosphate to proceed. A separate experiment measuring rSAP activity with a substrate by spectroscopy further confirmed the efficiency of heat inactivation. After only 5 minutes incubation at 65 °C or 1 minute at 75 °C there was no detectable rSAP activity (Figure 1B).

Uracil-DNA Glycosylase (UNG), Cod

Highly sensitive PCR and RT-qPCR have become routine assays in many life science labs, but they are unfortunately vulnerable to contamination from environmental DNA, and more significantly, to previous PCR products. Carry-over PCR contamination can cause false positive results, especially in labs continuously amplifying a particular set of targets. Guidelines for prevention of contamination, such as physical separation of sample preparation and amplification areas, have been well documented and are followed in most high throughput labs. As

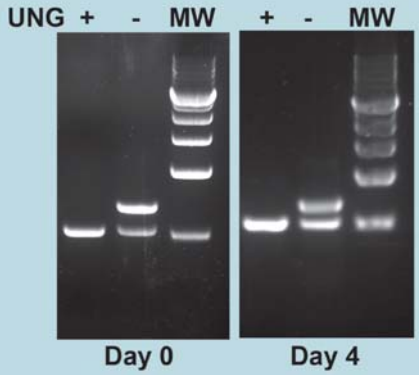


Figure 2. Uracil-DNA Glycosylase (UNG), Cod treatment specifically degrades dUTP-containing DNA in spiked PCR reactions and inactivates completely to allow subsequent synthesis of new dUTP-containing amplicons. Two PCR reactions were prepared with normal dTTP-containing DNA template and then spiked with a dUTP-containing amplicon from a previous PCR reaction. One PCR reaction was treated with UNG (1B1634) for 5 minutes at room temperature, while the other tube was left untreated. The treated and untreated samples were then immediately amplified by PCR, with the initial denaturation cycle serving as the heat inactivation step for the UNG treated sample. Following amplification, the PCR products with incorporated dUTP were analysed by gel. The amplicons were stored at room temperature for 4 days and then analysed by gel again. In the UNG treated sample, only one band was present due to effective degradation of the dUTP DNA. The dUTP DNA synthesised from dTTP template after UNG heat inactivation remained stable, even when stored at room temperature.

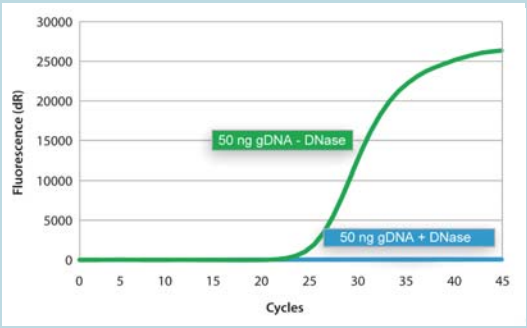


Figure 3A. Decontamination of a PCR master mix using DNase, Double-Strand Specific, Heat-Labile. A PCR master mix containing 50 ng genomic DNA was prepared and divided into two reactions, of which one was treated with DNase, Double-Strand Specific, Heat-Labile prior to qPCR of both samples. In contrast to the untreated sample, the DNase treated sample contained no amplifiable genomic DNA.

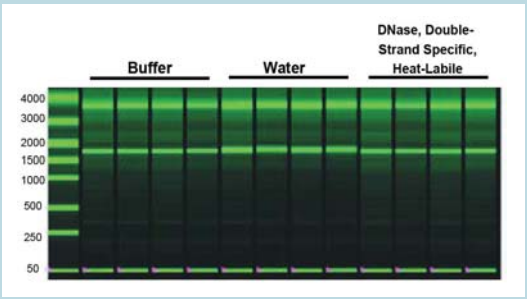


Figure 3B. DNase, Double-Strand Specific, Heat-Labile treatment leaves RNA quality intact. RNA incubated with buffer, water or DNase were analysed using the Eukaryote Total RNA StdSens Assay (Bio-Rad Experion System), with the results indicating all samples had intact, high quality RNA (RQI >8.5).

added precaution, it is also possible to specifically degrade carry-over PCR products using enzymatic digestion. AMRESCO's Uracil-DNA Glycosylase (UNG), Cod is a thermolabile recombinant enzyme that degrades uracil-containing single and double stranded DNA, but not RNA or thymidine containing DNA. The degradation occurs upon exposure to alkaline conditions and high temperature at sensitive sites generated by the hydrolysis of the N-glycosidic bond between deoxyribose sugar and the base in uracil. The prerequisite for UNG pre-treatment of PCR reactions is that dUTP be substituted in place of dTTP during all amplification reactions, such that all PCR amplicons can become substrates for UNG and all dTTP DNA will not.

In contrast to competing Uracil-DNA Glycosylases, AMRESCO's arctic-derived UNG has the distinct advantage of having a low inactivation temperature of 45 °C, which enables carry-over decontamination of one step reverse transcription reactions with UNG prior to the cDNA synthesis step. Inactivation is irreversible after heating just 20 minutes at 55 °C, or 1 second at 95 °C, allowing for stability of the cDNA that has incorporated dUTP. The irreversible inactivation feature of AMRESCO UNG also facilitates greater downstream manipulation and stability of newly synthesised dUTP-containing DNA by PCR, allowing for long-term storage, restriction digestion, cloning, sequencing and hybridisation applications. The gels in Figure 2 demonstrate the effective elimination of dUTP-containing DNA from spiked PCR samples that were treated or untreated with UNG for 5 minutes prior to PCR. The stability of the newly synthesised dUTP-containing amplicons after irreversible heat inactivation of UNG was assessed by gel analysis of the treated and untreated PCR samples after storage at room temperature for 4 days.

DNase, Double-Strand Specific, Heat-Labile

AMRESCO's DNase, Double-Strand Specific, Heat-Labile is an arctic-adapted recombinant endonuclease that cleaves phosphodiester bonds in DNA to yield 2 – 8 bp oligonucleotides with 5'-phosphate and 3'-hydroxyl termini. The high specific activity of this enzyme toward dsDNA can be inactivated by heating at 55 °C, conveniently eliminating the need for its physical or chemical removal before downstream processing, even in the presence of RNA and ssDNA, such as primers and probes. DNase, Double-Strand Specific, Heat-Labile treatment of samples allows for greater accuracy in the highly sensitive applications of RT-qPCR and qPCR because signals from unintended sources of dsDNA are eliminated. The DNase is ideal for removal of genomic DNA in RNA preps because its low inactivation temperature does not affect RNA stability and the reverse transcription reaction can be performed in the same tube. Removal of dsDNA carry-over contamination in PCR mixes before template addition works without any reliance upon dUTP substitution in prior PCR reactions, such as is required for UNG-based decontamination.

To demonstrate the activity of DNase, Double-Strand Specific, Heat-Labile, 50 ng of genomic DNA was added to a PCR master mix that was then divided into two separate reactions, with one untreated and the other treated with DNase. Only the DNase treated reaction amplified a detectable signal using an intron specific primer in qPCR (Figure 3A). DNase treatment was also performed on an RNA sample, which was analysed using the Eukaryote Total RNA StdSens Assay in parallel

VWR AMRESCO CHEMICALS

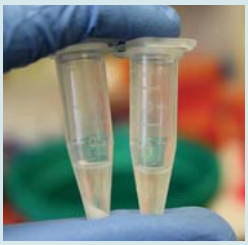


Figure 4A. SAN reduces viscosity in *E. coli* lysates. *E. coli* were lysed and then treated for 20 minutes at room temperature with (left) or without (right) SAN. The SAN treated lysate became less viscous than the untreated sample, and when both were centrifuged briefly, only the SAN treated sample formed a pellet. The untreated lysate was too viscous to pellet.

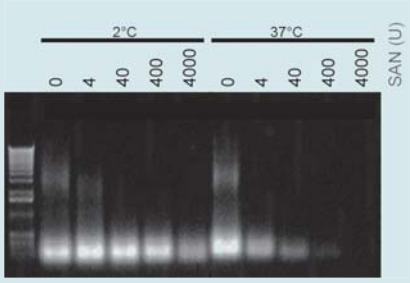


Figure 4B. SAN removes DNA in *E. coli* lysates. *E. coli* lysates containing 75 µg/ml DNA were resolved by agarose gel electrophoresis after 30 minute incubations at 2 °C or 37 °C with increasing units of SAN. The lysate buffer contained 50 mM Tris-HCl pH 8, 0.5 M NaCl, 0.1 mg/ml lysozyme and 10 mM $MgCl_2$. SAN activity was terminated by addition of EDTA. At both temperatures, SAN degraded the DNA into smaller fragments with increasing units of SAN.

with control RNA samples. The data show the quality and quantity of RNA remain intact after genomic DNA removal (Figure 3B). This was further demonstrated through RT-qPCR experiments comparing target amplification from cDNA synthesised from treated and untreated RNA (not shown).

During protein purification, high salt concentrations may be used to dissociate DNA protein complexes, thereby improving protein solubility and increasing accessibility of the DNA to nuclease activity. High salt conditions, however, are detrimental to most nucleases. An exception is AMRESCO's Salt Active Nuclease (SAN), which is optimised to work at low temperatures in moderate to high salt buffers. Its non specific endonuclease activity efficiently cleaves double- and single-stranded DNA and RNA into mostly 5-nucleotide oligos. SAN treatment of cell lysate also reduces sample viscosity, which is problematic for downstream filtration and centrifugation steps in the protein purification process. Removal of SAN activity following treatment is achieved by a combination of heating and addition of a reducing agent, such as DTT. SAN may also be physically removed by ion exchange chromatography.

The effect of SAN treatment on viscosity of a protein lysate can be seen in Figure 4A, with the treated and untreated samples on the left and right, respectively. Upon brief centrifugation, only the SAN treated lysate could form a pellet of debris, because the untreated lysate was too viscous. Figure 4B demonstrates the nuclease activity of SAN in high salt lysis buffer at both low and high temperatures. DNA removal in *E. coli* protein lysates was monitored by agarose gel electrophoresis following a 30 minute incubation with increasing units of SAN at 2 °C or 37 °C. At both temperatures, SAN degraded DNA into smaller fragments with increasing units of SAN.

VWR COLLECTION

VWR® PCR Workstation

VWR INTRODUCES THE NEW LABORATORY STANDARD

The brand new VWR PCR Workstation is designed as an ideal environment for the manipulation of DNA and RNA, especially for the setup of PCR assays. Contaminations can lead to false or misleading results which costs time and money.
The VWR PCR Workstation minimizes the risk of contamination. It provides a 'separated room', e.g. for the setup of PCR reactions. The high intensity surface UV tubes inactivate DNA as a source of contamination between experiments. In addition, an UV Air Recirculator is integrated into the workstation system which reduces airborne microbes and contaminants during experiments. UV light is effectively blocked by solid polycarbonate screens to ensure maximum protection of the user.
The inner surfaces of the workstation are made of stainless steel, which is very robust, can easily be cleaned and has an antimicrobial effect.
The VWR PCR Workstation offers a controlled environment for PCR and RNA applications that protects your precious samples and helps to achieve optimal results.

OUTSTANDING FEATURES

- 2 powerful 25 W 254 nm UV-tubes for efficient surface decontamination before or after experiments
- Programmable timer for automated execution of daily decontamination routines
- UV air recirculator for continuous air decontamination during work
- Stainless steel surfaces: easy to clean, antimicrobial, long lasting
- Large front door allows easy and convenient access of work space
- Ethanol resistant, very robust Makrolon® screens for full protection from UV light
- overflow protection: spilled chemicals can't run out of the workstation
- Counter of UV runtime for timely exchange of UV tubes to maintain constant intensity
- Very easy assembly and installation

Technical data	
Timer UV light:	5 -30 min, progammable for daily routines
Front and side panels:	8 mm Makrolon® protects from UV irradiation
Housing, surface:	Stainless steel
Work area:	720 x 540 mm (L xD)
Outer dimensions:	750 x 780 x 620 mm (L x H x D)

Description	Cat. No.
PCR workstation with UV air recirculator	732-2541
PCR workstation with UV air recirculator, UK version	732-2542

Not available in Germany and Austria

The large working area, removable shelves and 4 power outlets provide ample space for comfortable operation and allow the combination of several working steps protected from contamination.

GE Healthcare Life Sciences launches SimpliNano, easy to use, accurate low volume spectrophotometer

Distributor
GE Healthcare

Figure 1. SimpliNano is an easy to use, low maintenance spectrophotometer designed for analysis of low volumes of nucleic acid and protein samples, shown here with printer option.

No calibration required to accurately quantitate small volumes of nucleic acids and proteins

SimpliNano is a simple to use microvolume spectrophotometer for straightforward concentration and purity measurements of nucleic acids and proteins (Figure 1).

It uses a built-in sample port that can be used with a variety of common lab chemicals and has no moving parts, making it reliable, easy to use and clean. It has a fixed path length that does not require any calibration. The ability to pipette volumes down to 1 µl directly onto the sample port eliminates the need for cuvettes or other sampling tools. After measurement, the sample can either be easily recovered with a pipette or quickly discarded by wiping the sample port clean for the next measurement.

SimpliNano is pre-programmed with a range of pre-set methods for the quantitation of nucleic acids and direct UV proteins, with graphical display of resulting wave scans. The SimpliNano direct user interface means that there is no need for a computer, so fast instrument start-up is possible.

Assays

Nucleic acids

SimpliNano allows you to measure the concentration and purity of nucleic acids in a variety of units (µg/ml, ng/µl, and µg/µl,) and also, to correct for dilution factors where necessary. The spectrophotometer displays both individual absorbance values and absorbance ratios (260/280 and 260/230) on the screen, along with the sample concentration value. The results can be printed immediately with the on-board printer option. The pre-stored method includes the option of background correction at 320 nm. You can examine the UV spectrum (220 to 320 nm) for hybridisation, PCR and sequencing studies or for quantitation of mini preps.

SimpliNano shows excellent linearity with double-stranded DNA (dsDNA) from 4 to

Figure 2. The built-in sample port allows direct application of 1 to 5 µl volumes of sample.

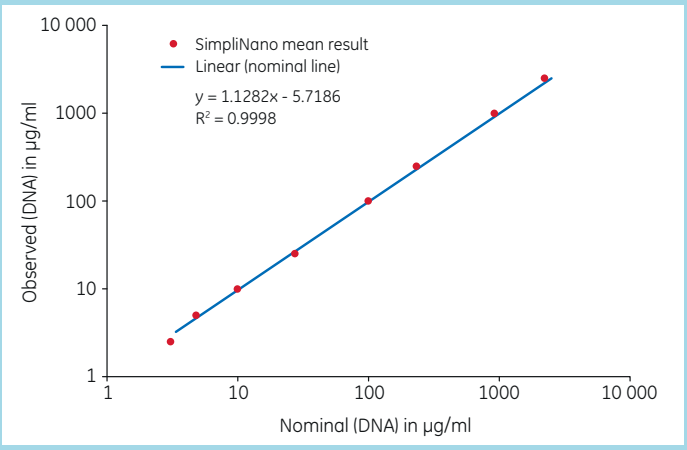


Figure 3. DNA concentration curve (4 to 2500 µg/ml) generated from 10 replicate salmon sperm dsDNA samples (2 µl volumes) measured at 260 nm on SimpliNano. The data in the graph is representative data from one instrument used in the validation study where a total of 6 instruments were tested.

SimpliNano offers the following benefits:

- **Simple sample handling:** Built-in sample port (Figure 2) with one fixed path length means that samples can be added and removed without the need for time-consuming path length calibration. SimpliNano contains no moving parts, which contributes to excellent instrument reproducibility and low maintenance.
- **Low sample volumes:** Reduce sample loss and eliminate the need for dilution by using low volumes of 1 to 5 µl for sample measurement. The sample can either be recovered or simply wiped away after measurement.
- **Built-in life science methods:** Measures nucleic acid concentrations at 260 nm, protein concentrations at 280 nm and provides 230/260 and 260/280 ratios for determining sample purity. Wave scan plots from 220 to 330 nm can be achieved for nucleic acids and from 250 to 350 nm for protein. The methods include options for background correction at 320 and 340 nm.
- **Flexible instrument control:** Use as either a stand-alone instrument using its large, built-in screen for quick measurements or operate from a PC with optional Datrys software.
- **Choice of data output:** Output to any suitable PC via a USB cable, to an optional integrated printer, or to a USB stick.

2500 µg/ml (Figure 3). This large linear dynamic range and high reproducibility means that you can proceed to the next stage of your workflow with a high degree of confidence in the data.

Proteins

SimpliNano can be used to determine the concentration of protein samples in the near UV spectrum at 280 nm due to absorption by the amino acids tyrosine, tryptophan, and phenylalanine. The protein method shows individual absorbance readings for 260 and 280 nm and offers background correction at 340 nm. A 260/280 ratio is also provided on the screen. The absorbance at 280 nm varies greatly for different proteins because of their amino acid content and therefore, the specific absorption value for a particular protein must always be determined. SimpliNano has built-in extinction coefficients for BSA, IgG, and lysozyme, allowing you to select the most appropriate values for your protein sample. You can also input your own extinction coefficients. Figure 4 shows protein linearity over the range of 0.12 to 50 mg/ml.

Sample can be simply removed with a tissue to prevent cross-contamination thus improving data precision. To demonstrate this, sample carryover was determined using high concentrations of protein (BSA) and the results of the carryover experiments are shown in Figure 5.

Conclusion

SimpliNano spectrophotometer is an easy to use and reliable instrument for measuring nucleic acid and protein samples, without the need for path length calibration. SimpliNano offers wave scan plots for nucleic acids and proteins. Samples of 1 to 5 µl can be pipetted directly onto the sample port for measurement, and then simply recovered using a pipette. In cases where sample recovery is not necessary, the sample port can be quickly and easily wiped clean. This eliminates the need for cuvettes, capillaries, or other sampling tools— just drop, measure and you're done.

Description	Cat. No.
SimpliNano (worldwide except USA and Canada)	29-0617-11
SimpliNano with printer (worldwide except USA and Canada)	29-0617-12

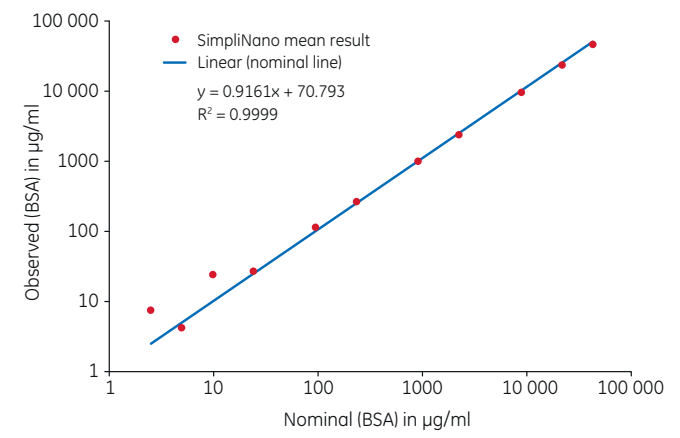


Figure 4. BSA concentration curve (120 to 50 000 µg/ml) generated from 10% BSA solution. Ten replicate samples (2 µl volumes) were measured at 280 nm on SimpliNano using the BSA program to ensure correct protein coefficient was employed. The data in the graph is representative data from one instrument used in the validation study where a total of 6 instruments were tested.

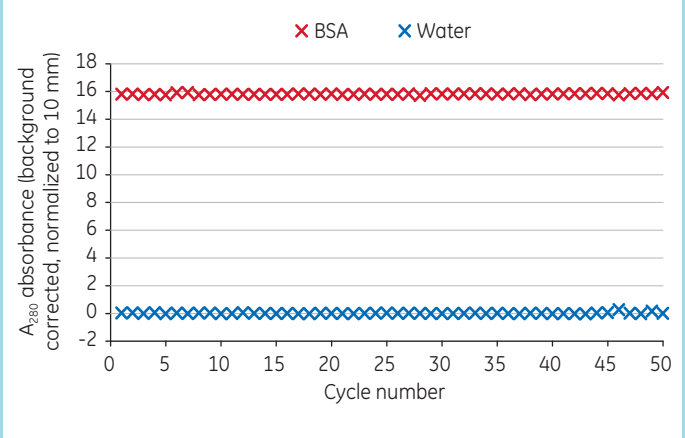


Figure 5. A sample carryover study using 25 mg/ml BSA with successive alternative additions of water and BSA, which was repeated 50 times. The data showed no significant change in absorbance readings throughout the study for the water samples or the BSA samples, indicating that there was no carryover.

DNA quantification in microlitre volumes

UV photometry is a common way to quantify nucleic acids in a sample.

Both DNA and RNA absorb UV light very efficiently, making it possible to detect and quantify the concentrations. Typical applications for this include, for example, the quantification of template prior to sequencing or PCR.

With Thermo Scientific µDrop Plate it is possible to measure DNA concentrations from a few nanograms to thousands of nanograms per microlitre.

The fixed path length of the plate enables direct calculation of the nucleic acid concentrations.

INTRODUCTION

The photometric method is based on Lambert-Beer's equation and it is based on fact that the nitrogenous bases in nucleotides have an absorption maximum at about 260 nm.

For dsDNA 1,0 Abs at 260 nm corresponds to a concentration of 50 µg/ml. As a result the amount of DNA can be calculated by using the formula:

DNA concentration (µg/ml) =
Abs 260 x 50 µg/ml

Unlike the nucleic acids, proteins have a UV absorption maximum at 280 nm, mostly due to the tryptophan residues. Therefore, the Abs_{260}/Abs_{280} ratio gives an estimate of the protein contamination of the sample. For a good quality sample, the value should be between 1,8 and 2,0. A value smaller than 1,8 indicates the presence of proteins and a value higher than 2,0 indicates probable contamination, such as phenols.

Another common parameter used to describe the quality of DNA is the 260 to 230 nm ratio. It is used to estimate chemical contamination, such as phenols, carbohydrates or a high salt concentration. The ideal 260/230 nm ratio is around 2.

A possible background caused by impurities in the sample can be corrected by a measurement made at a wavelength, at which the absorption level for nucleic acids and proteins is really low. The wavelength most commonly used for this background subtraction is 320 nm; (Abs_{260}-Abs_{320} / Abs_{280}-Abs_{320}). For example, magnetic beads are now commonly used in the nucleic acid purification process, and when bead-based purification is used, the 320 nm subtraction is always recommended.

Figure 1. µDrop plate and Multiskan GO spectrophotometer. The low sample volume measurement area with 16 measurement locations on the left and a cuvette location on the right part of the plate.

Thermo SCIENTIFIC

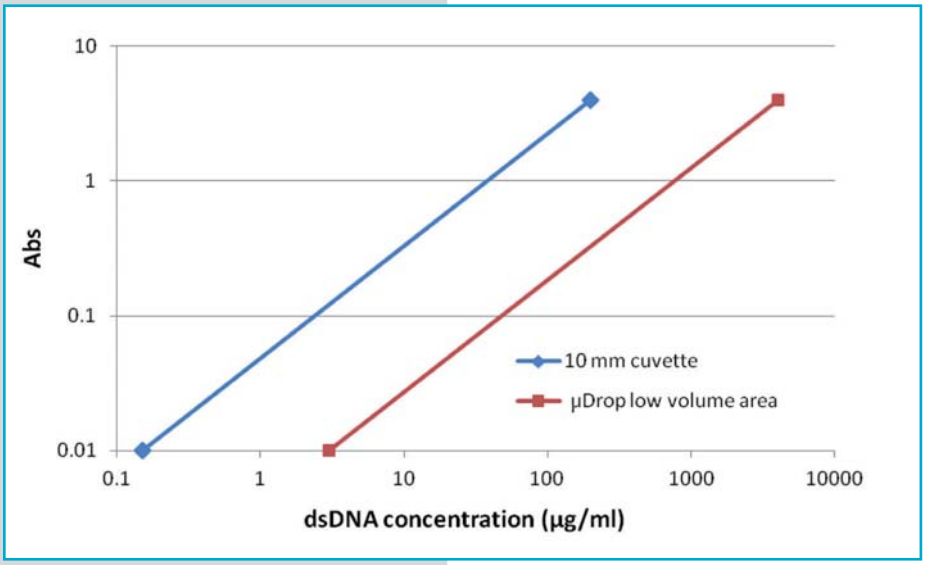


Figure 2. The theoretical difference between the detection ranges of the cuvette and µDrop Plate.

With a µDrop Plate and a photometer with high precision and a wide linear range, it is possible to measure DNA concentrations from a few nanograms to thousands of nanograms per microlitre.

µDrop plate is compatible with Thermo Scientific Multiskan GO microplate spectrophotometer and Thermo Scientific Varioskan Flash multimode reader.

The amount of sample available for the analysis is quite often really low, as a result there is a need for a tool such as the µDrop™ Plate that enables these measurements at a microlitre scale.
The µDrop Plate consists of two separate measurement locations, one for measuring low sample volumes and the other for cuvettes (Figure 1).

The low volume measurement area consists of two quartz slides, the top clear quartz slide and the bottom partially Teflon®-coated quartz slide. The bottom slide contains 16 sample positions, arranged in a 2 x 8 matrix, onto which samples can be pipetted. The cuvette slot of the plate is used to perform photometric measurements with standard cuvettes.

BLANK

Any photometric measurement device, cuvette, microplate or µDrop Plate, always has certain background absorption. Therefore, blank subtraction is always necessary when photometric quantification of the sample concentrations is performed.

PATH LENGTH

Path length of the µDrop Plate is the distance between the quartz glass surfaces, i.e. the length of the light beam in the liquid sample.
Absorbance always depends on the path length and when pathlenght is shorter, it is possible to measure bigger concentrations.

Therefore it is possible to measure higher concentrations with the µDrop plate than with e.g. a 10 mm cuvette.

DETECTION RANGE

With a µDrop Plate type of a measurement device, the maximum measurement range is always determined by the instrument. The lower part is determined by the precision of the blank (LOD) and the upper part by the linear range of the instrument.

A shorter path length also increases the requirements of the photometer used as it reduces the measured absorbance. For photometers the upper part of the absorbance area (high concentrations) is especially difficult, because less and less photons reach the detector.

Both the theoretical minimum and maximum concentrations that can be measured on the µDrop Plate are instrument dependent and can be calculated from the given instrument specifications. For example, with Multiskan GO, which has a given precision specification of 0,003 Abs and a linearity up to 2,5 Abs, the theoretical maximum concentration range is from 3 x 0,003 x 50 µg/ml x (10 mm/0,5 mm) **= 9 µg/ml** to 2,5 x 50 µg/ml x (10 mm/0,5 mm) **= 2500 µg/ml**

A theoretical comparison of a dsDNA measurement with a 10 mm cuvette and the µDrop Plate low volume area with an instrument with the performance described above is shown on Figure 2.

AppliChem's solution!

Nucleic acid decontamination with DNA-ExitusPlus™

DNA contamination of PCR workstations leads to faulty DNA amplifications. Hidden traces of DNA may cause false diagnoses or contamination of sequence databases. Control and elimination of unwanted DNA background therefore is mandatory for quality control in all PCR laboratories.

Due to the extremely high sensitivity of current detection methods, free nucleic acids (originating from preparations of DNA or RNA from viruses, bacteria, fungi, plants or any other eukaryotes) may cause severe contamination problems in research labs, in molecular biology analysis, in clinical diagnostics and in forensic analysis. The complete decontamination of equipment and surfaces from any DNA is one important step forward to biological containment and safety, as well as preventing misleading PCR results. AppliChem's patented product DNA-ExitusPlus™ employs a mild and non-corrosive chemistry for rapid non-enzymatic degradation of nucleic acids. Even a short incubation time with DNA-ExitusPlus™ completely removes non target DNA and RNA from work surfaces and tools [1, 2].

There are two different versions of DNA-ExitusPlus™ available: DNA-ExitusPlus™ (A7089) includes a colour indicator to easily see the surface area covered by the reagent. DNA-ExitusPlus™ IF (A7409) is almost colourless. Both variants share their outstanding and unique characteristics:

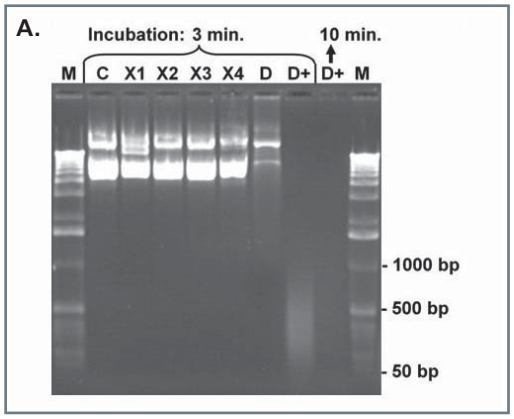
I. **A very rapid non enzymatic, non sequence specific degradation of DNA and RNA molecules caused by a catalytic and cooperative effect of all ingredients.**

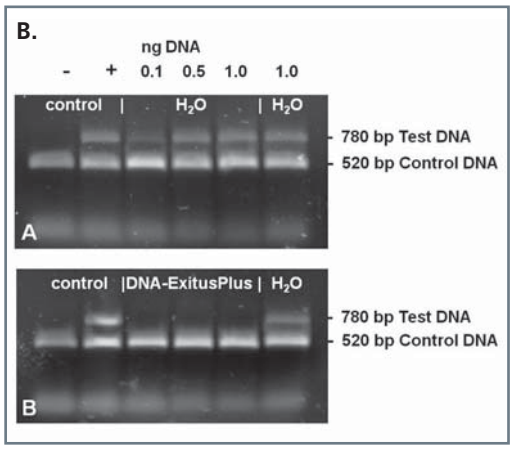
II. **No aggressive mineral acids or alkaline substances are used. Equipment and materials are not damaged or corroded even after prolonged incubation times.**

III. **All components of DNA-ExitusPlus™ are readily biodegradable and not harmful or toxic for humans. No harmful aerosols are formed when spraying on surfaces.**

The DNA- ExitusPlus™ technology is the first choice for:

- **Removing DNA and RNA contaminations from surfaces**
- **Decontaminating of PCR work stations and equipment**
- **Cleaning of pipettes, etc.**
  DNA-ExitusPlus™ does not damage your valuable equipment - in contrast to classic nucleic acid decontamination products that are based on aggressive mineralic acids (such as phosphoric acid or hydrochloric acid), bases (e.g. NaOH and KOH), sodium hypochlorite ("bleach") or H_2O2.





Verify the effectiveness of decontamination agents

It's harder than it seems to test decontamination solutions for a complete DNA degrading activity. It is not sufficient at all to just mix decontamination solution and DNA and to perform a subsequent PCR to amplify residual DNA. Since PCR is too easily affected by factors such as unfavourable buffer conditions, PCR inhibitors or DNA cross contamination.
But for the evaluation of the potential of a DNA decontamination reagent, one has to use PCR analysis in combination with a sensitive DNA degradation test. We have developed a DNA strand break assay which allows us to evaluate the efficiency of DNA-ExitusPlus™ [1].

A. The DNA strand break assay in brief:

1. Incubate for 3 - 10 min 200 ng of plasmid DNA with water (C), commercial decontamination solutions (X1-X4) and DNA-ExitusPlus™ (D+).
2. Denature all samples with heat.
3. Agarose gel electrophoresis of DNA and degradation products.

Results: While the control treatment and all tested competitors show no DNA degradation, plasmid DNA is degraded completely after 10 min of treatment with DNA-ExitusPlus™. 3 min of DNA-ExitusPlus™ treatment leads already to a strong fragmentation of the test plasmids.

B. The PCR test in brief:

1 Incubate 0.1 to 1 ng of test DNA with water (A) or DNA-ExitusPlus™ (B).
2. Wash twice the reactions.
3. Add a control DNA, and
4. Run PCR with primers against test and control DNA.
5. Agarose gel electrophoresis of PCR products.

Results: After incubation with DNA-ExitusPlus no PCR amplicons for the test DNA can be detected indicating all test DNA template has been degraded. The PCR product for control DNA confirms functionality of the PCR.

Products for nucleic acid decontamination		
Description	**Pk**	**Cat. No.**
DNA-ExitusPlus™	100 ml	A7089.0100
	250 ml	A7089.0250
	500 ml	A7089.0500
	1 l	A7089.1000
DNA-ExitusPlus™ IF	100 ml	A7409.0100
	250 ml	A7409.0250
	500 ml	A7409.0500
	1 l	A7409.1000
Derma-ExitusPlus™ for the skin, hands (or gloves)	250 ml	A8740.0250
Autoclave-ExitusPlus™ additive to clear cell culture waste	Powder for 6x 1 l	A7600.1000

DNA-ExitusPlus™ is a registered trademark of AppliChem.

Literature

1) Esser et al. (2006) DNA Decontamination: Novel DNA-ExitusPlus™ in comparison with conventional reagents. BioTechniques 40 (2), 238-9. – On closer inspection, only DNA-ExitusPlus™ destroys DNA completely.

2) Arena, A. (2010) Dna Exitus Plus™ Versus Standard Bleach Solution for the Removal of Dna Contaminants on Work Surfaces and Tools. Investigative Science Journal 2, 20-9. – DNA-ExitusPlus™ proved as effective as freshly prepared bleach solution in an assay using swap tests and subsequent qPCR.

FectoPRO™

Achieve amazing protein yields in CHO and HEK-293 cells

Transient gene expression (TGE) is commonly used for medium scale production of recombinant proteins and antibodies. This approach allows generation of sufficient protein amounts avoiding a major investment in production of stable cell lines prior to "proof of concept" studies or tools validation. Indeed, the speed and flexibility of TGE has enabled this technique to be widely adopted in bioproduction for early discovery, research applications and process developments.

However, the protein yields usually remain lower with TGE than with stable gene expression process. In order to improve your productivity, Polyplus-transfection has developed a novel transfection kit to generate superior protein amounts by TGE for medium scale bioproduction.

FectoPRO™

FectoPRO™ is a novel powerful solution for improved protein yields in TGE systems. This kit was designed after extensive screening of numerous chemical structures based on their transfection efficiency, protein production yield and cell viability. It is suitable for transfection of suspension CHO and HEK-293 cells in various serum-free media, using low DNA amount (<1 µg/ml of cell culture).

FectoPRO™ outperforms other currently available transfection reagents such as PEIs or lipid-based reagents and shows unprecedented results in both CHO and HEK-293 cells.

Transfection efficiency

We compared FectoPRO™ with other commercially available transfection reagents and measured the obtained transfection efficiency. FectoPRO™ in combination with its booster gave by far the highest transfection efficiency in both suspension CHO and HEK-293 cells. (Fig.1)

Protein yields

Productivity was assessed by production of an IgG fragment. In both CHO and HEK-293 cells, FectoPRO™ largely outperforms other transfection reagents commonly used in bioproduction processes in terms of protein yields. (Fig.2)

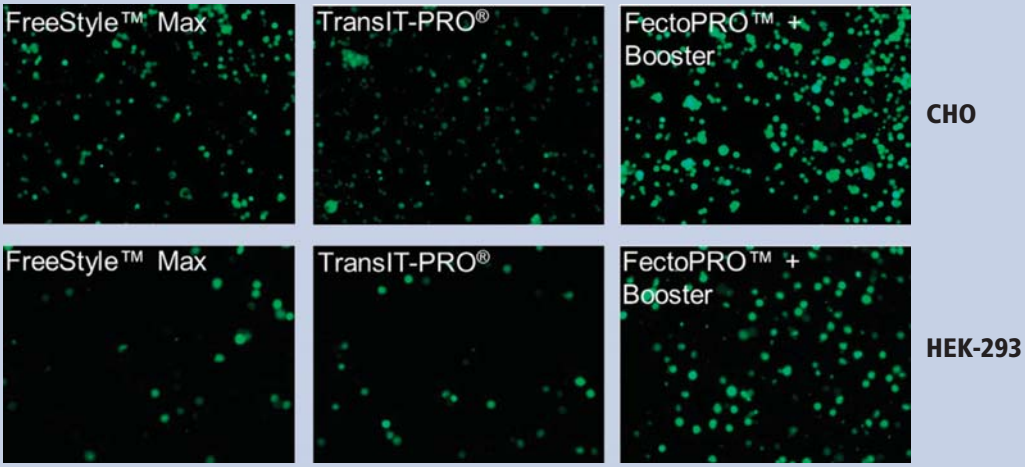


Figure 1. FectoPRO™ outperforms the competitor reagents and achieves highest transfection efficiency. FreeStyle™ CHO-S and 293-F cells were seeded at 1x 106 cells/ml in 30 ml of their recommended FreeStyle™ Media and transfected using a GFP expressing plasmid with FreeStyle™ MAX Reagent (1.25 µg/ml), TransIT-PRO® (1 µg DNA/ml) or FectoPRO™ + FectoPRO™ Booster (0.5 µg/ml).GFP expression was measured 24 h after transfection using fluorescence microscopy.

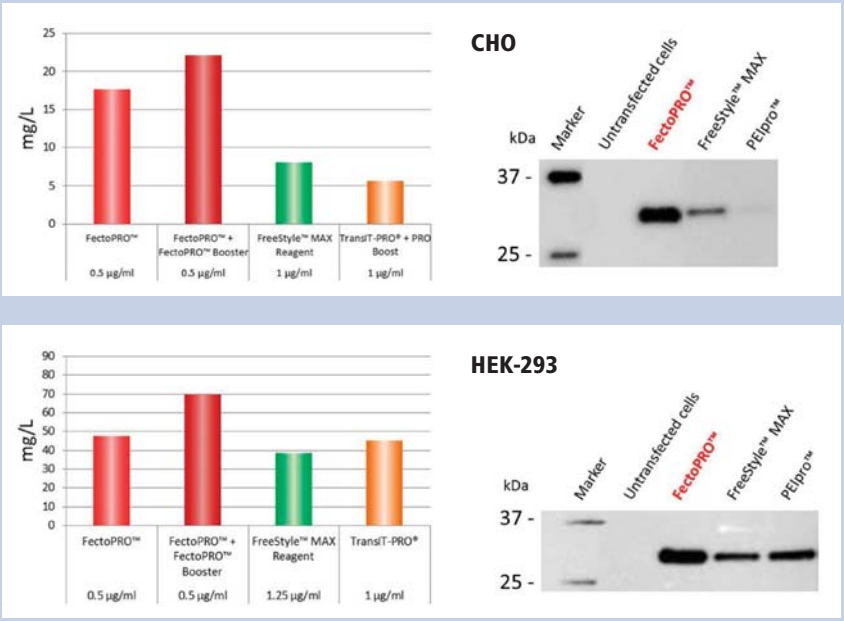


Figure 2. FectoPRO™ leads to unprecedented protein yields in TGE. CHO and HEK-293 cells were transfected with a IgG3-Fc expressing plasmid using reagents and conditions mentioned. Antibody quantitation was performed by using protein G affinity column (HPLC) and qualitative analysis was done by Western Blot 120 hours post transfection.

Reagent	DNA amount µg/ml cell culture	Reagent volume µl/ml cell culture
FectoPRO™ + FectoPRO™ Booster	**0.4 - 0.6**	**0.6 - 0.9**
FreeStyle™ MAX Reagent	1.25	1.25

Table 1. Amount of DNA and volume of reagent needed for transfection according to manufacturers' recommendations.

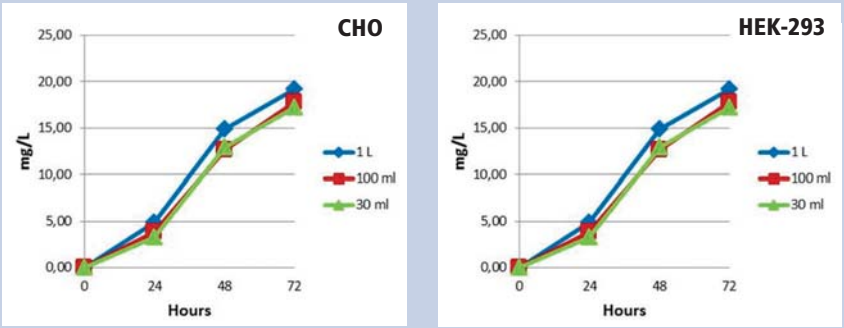


Figure 3. FectoPRO™ is easily scalable and reproducible. IgG3-Fc production in different volumes of cell culture after transfection with DNA/FectoPRO™ (0.4 µg/0.72 µl) and Booster (0.25 µl) per ml of CHO cell culture and with DNA/FectoPRO™ (0.5 µg/0.75 µl) and Booster (0.30 µl) per ml of HEK-293 cell culture. Quantitation was performed by using protein G affinity column (HPLC).

Cost effective transient gene expression

High quality plasmid DNA preparation can be very costly, especially when large DNA amounts are required for large scale protein production. FectoPRO™ saves on DNA cost by using only 0.4 to 0.6 µg DNA/106 cells/ml of cell culture making this kit an affordable and economical solution for bioproduction processes. (Table 1)

Highly scalable

FectoPRO™-mediated transfection process is easily scalable from a few millilitres to several litres of cell culture, ensuring robust reproducible protein production. FectoPRO™ simplifies your bioproduction process with a robust protocol that is easily adapted to different culture vessels. (Fig.3)

Compliant with biomanufacturing guidelines

FectoPRO™ is chemically defined and guaranteed free of animal-origin components. Systematic lot management and release testing is performed for each lot produced. FectoPRO™ transfection kit undergoes advanced quality controls for protein productivity, cell viability and complete sterility.

In addition, Polyplus-transfection® is ISO 9001 Quality Management System accredited since 2002. This level of certification ensures our customers worldwide that we have established reliable and effective processes for product development, manufacturing, sales and customer support.

Introductory offer for 1 ml FectoPRO™ available, please contact your local VWR sales office.

Volume of FectoPRO™ Reagent	Volume of FectoPRO™ Booster	Cat. No.
1 ml	1 ml	116-001
10 ml	10 ml	116-010
10x 10 ml	10x 10 ml	116-100

FreeStyle™ is a trademark of Life Technologies™ Corporation
TransIT-PRO® is a registered trademark of Mirus Bio LLC.

OMEGA bio-tek

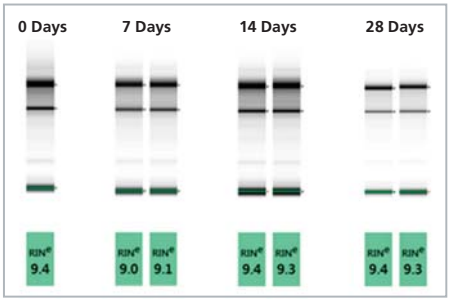
As generating expression profiles with Next Generation sequencing becomes more popular, the need for a reliable method to transport purified RNA is paramount. It is increasingly common for collaboration among multiple sites where RNA is purified at one location and analysed at another. Current methods include overnight shipments on dry ice or drying samples into a gel matrix.

Room temperature storage and shipping of purified RNA

Transporting purified RNA offers unique challenges as the sample can become degrade during shipment, in preparation for shipment, in transit, or during the receiving process. During transit, samples can also degrade due to carrier delays, samples becoming displaced from the dry ice, or dry ice evaporation. With these conditions in mind, Omega Bio-tek has developed RNA Transport to eliminate dry ice shipments allowing for samples to be shipped via ground transportation at significant cost savings while maintaining RNA quality.

EFFECTS OF ROOM TEMPERATURE STORAGE WITH RNA TRANSPORT

Total RNA was purified from cultured cells using the E.Z.N.A. Total RNA Kit 1. 4 µg RNA was aliquoted to individual tubes containing RNA Transport then stored at room temperature for the indicated days. RNA was repu¬rified using RNA Transport purification method which takes less than 5 minutes per sample set. RNA quality was analysed via Agilent Tapestation 2200.



To stabilise purified RNA, samples simply need to be mixed with RNA Transport. No long tedious lyphilization steps or creating a gel matrix, in less than 10 seconds your RNA sample is protected. Once received, RNA samples can be left at room temperature until the receiving site is ready to process them, thus eliminating the need for -80 °C storage.

RNA TRANSPORT WORKFLOW

Add sample to vial containing RNA Transport and vortex for 30 seconds
Ship via Ground or Air

Recovery procedure
- Add 80% ethanol to sample and apply to silica column
- Wash with 80% ethanol
- Dry
- Elute

Save on shipping costs
RNA Transport eliminates the need for cost bulk boxes, dry ice and Styrofoam containers and overnight shipping. The bulky packaging requirements for dry ice increases shipping charges and usually require an overnight service or, with international shipments, that dry ice be replenished. RNA Transport is supplied in a 12 mm, screw cap, 2.0 ml vials for ease of use and reduction of shipping costs.

Cost of shipping RNA samples
Cost analysis of shipping dry ice packages overnight versus shipping in smaller packages via ground shipments.

Automation
For core facilities and sequencing centres a common problem is receiving samples in various plasticware which is not compatible with automation or does not match existing labware definitions. Having customers using RNA Transport allows for your procedures to be standardised and sample transfer to be automated with liquid handling instruments with individual probes such as Hamilton STAR/STARlet, Tecan Evo, and Beckman Coulter Biomek FX/NX.

Flexibility
RNA Transport allows customers to ship samples any day of the week instead of limiting shipments from Monday to Wednesday. Even in extreme temperatures during winter and summer months, RNA

Thermo SCIENTIFIC

Peptide-Oligonucleotide-Conjugates

Peptide-Oligonucleotide conjugate (POC) are composed of a single strand DNA Oligonucleotide which is covalently bound to a short peptide sequence. Most recently POCs have been used in numerous applications from therapeutics to nanotechnology. Whereas in the past these conjugates became useful especially to lead and facilitate the transport of nucleic acids into cellular compartments by using cellular translocation signals [2, 3, 4], today they become also interesting as combinatorial agents in the assembly of bivalent protein affinity reagents [5].

Description of the conjugation

Molecules, where the N- or C-terminus of a peptide is covalently linked to the 3'- or 5'-terminus of an oligonucleotide, resulting in general, in a linear peptide-oligonucleotide-conjugate. The standard reagent for conjugation is the heterobifunctional crosslinking reagent succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), which enables amine-modified oligonucleotides to become covalently linked to cysteine-modified polypeptides [5]. A second method using the same reagent is coupling the N-terminal amino group of the peptide to the terminal thiol group of the oligonucloetide.

Advantages

- Facilitate transport of nucleic acids through cell membranes
- Enable targeted transport of nucleic acids to cellular compartments (cytoplasm, nucleus, etc) by using cellular translocation signals
- Bi-functionality

Applications

- Anti-sense or gene silencing experiments
- in-situ hybridisation
- Targeted direction of nucleic acids into cells

Literature

1. *Antopolsky M. and Azhayev A., (1999) Stepwise Solid-Phase Synthesis of Peptide-Oligonucleotide Conjugates on New Solid Supports. Helvetica Chimica Acta, Vol. 82, pp. 2130-2140.*
2. *Zanta M.A., Belguise-Valladier P., Behr J-P., (1999) Gene delivery: A single nuclear localization signal peptide is sufficient to carry DNA to the cell nucleus. PNAS Vol. 96, pp. 91-96.*
3. *Richard J. et.al., (2003) Cell-penetrating peptides – A reevaluation of the mechanism of cellular uptake. J Biol Chem, Vol. 278, No. 1, pp. 585-590.*
4. *Moulton HM. and Moulton JD. (2003) Peptide-assisted delivery of steric-blocking antisense oligomers. Curr Opin Mol Ther. 5(2), pp. 123-132.*
5. *Berea A.R. Williams and John C. Chaput (2010) Synthesis of Peptide-Oligonucleotide Conjugates Using a Heterobifunctional Crosslinker; Curr Protoc Nucleic Acid Chem. 2010 September ; CHAPTER: Unit4.41. doi:10.1002/0471142.*

Please contact your local VWR sales representative for an individual quotation.

Standard peptide-oligonucleotide-conjugates are HPLC-purified and consist of:

	Peptide	Oligonucleotide
Length	6 - 15 amino acids*	8 – 40 DNA bases*
Purity	>95%	HPLC-purified
Coupling via	N- or C-terminal Cys N-terminal amino group	3'- or 5'-terminal aminolink 3'- or 5'-terminal thiol link
Restrictions	Only one Cys per peptide allowed! No internal Lys allowed	None
Modifications	Please enquire	Combination possible with: - PTO bases - Additional dye modification (such as Fluorescein or Cy3)

**Longer sequences are available on request.*

Standard peptide-oligonucleotide-conjugates are available in the following synthesis scales:

Synthesis scale	0.02 µmol	0.04 µmol	0.2 µmol	1.0 µmol
Conjugate yield in OD*	1	2	5	10

**Optical density at λ= 260 nm.*

Transport is able to protect samples for use in downstream applications.

International customers have the added security that if samples are delayed in customs that the RNA is still intact and safe.

Variable temperatures	
Sample	**RIN value**
Original sample	9.4
2x Freeze Thaw A	9.1
2x Freeze Thaw B	9.0
37 0C for 7 days A	8.4
37 0C for 7 days B	8.5

Total RNA was purified from cultured cells using the E.Z.N.A. Total RNA Kit 1.4 µg RNA was aliquoted to individual tubes containing RNA Transport then stored at -20 °C for 24 hours, thawed at room temperature for 24 hours for the indicated days. RNA was repurified using RNA Transport purification method which takes less than 5 minutes per sample set. RNA quality was analysed via Agilent Tapestation 2200.

Description	Size	Cat. No.
RNA Transport	5 preps	R0527-00
RNA Transport	50 preps	R0527-01
E.Z.N.A. Total RNA Kit1	50 preps	R6834-01
E.Z.N.A. Total RNA Kit 1	200 preps	R6834-02
HiBind RNA Mini Columns	50 preps	RNACOL-01
HiBind RNA Mini Columns	200 preps	RNACOL-02

NucView™ Caspase-3 Substrates for real

Now available in two colours for more applications.

Proteolysis of cellular substrates by caspase-3 results in the morphological and biochemical features of apoptosis. NucView™ Caspase-3 Substrates are novel fluorogenic caspase substrates designed for detecting caspase-3 activity in real time in intact cells.

Traditional fluorogenic caspase substrates require cell lysis and cannot be used to measure caspase activity in live cells; furthermore such assays measure only the average caspase activity in a cell population. Fluorescently-labelled caspase inhibitor assay (FLICA) reagents can enter live cells to detect caspase activity, but because the fluorescent probes are irreversible caspase inhibitors, they cannot be used to follow caspase activity in real time.

Biotium scientists have invented a novel fluorogenic enzyme substrate design by attaching an enzyme substrate moiety to a nucleic acid dye. In the case of NucView™Caspase-3 Substrates, the NucView™ DNA dye is attached to the caspase-3/7 substrate peptide sequence DEVD (Cen et al. 2008). Once linked to the substrate peptide, the dye is unable to bind to DNA and remains non fluorescent. The substrate enters the cytoplasm where it can be cleaved by caspase-3/7 in apoptotic cells. Caspase cleavage of the substrate releases the high affinity fluorescent DNA dye, which stains the cell nucleus with bright and stable fluorescence signal (Figure 1). Blue fluorogenic NucView™ 405 Caspase-3 substrate is designed for excitation with the 405 nm violet laser (Figure 2A), while green fluorogenic NucView™ 488 Caspase-3 Substrate can be detected using the same instrument settings as FITC (Figure 2B).

NucView™ substrates do not interfere with caspase-3 activity, allowing real time monitoring of fluorescence kinetics over time frames of hours to days. Caspase-3 detection can be performed after incubating cells with substrate for just 15 minutes in cell culture medium with no washing required, making it compatible with high throughput screening and high content analysis. After staining, cells can be fixed in formaldehyde and processed for subsequent immunostaining. Detection of caspase-3 activity using green fluorogenic

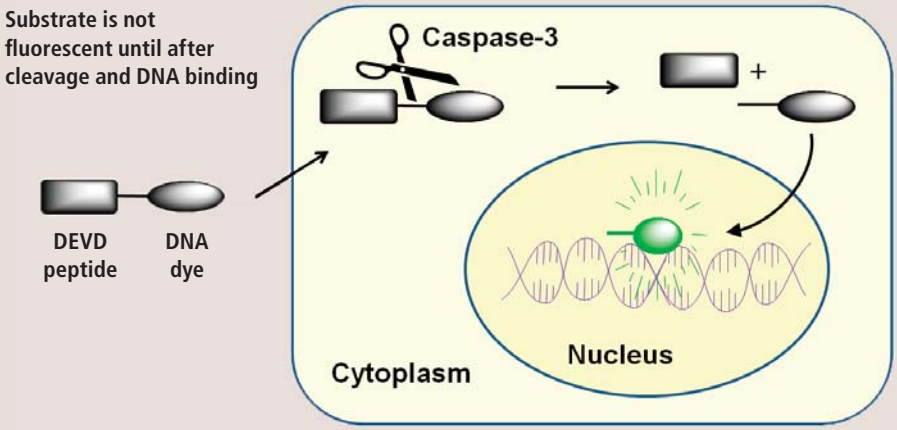


Figure 1. Schematic illustrating the mechanism of NucView™ Caspase-3 Substrates.

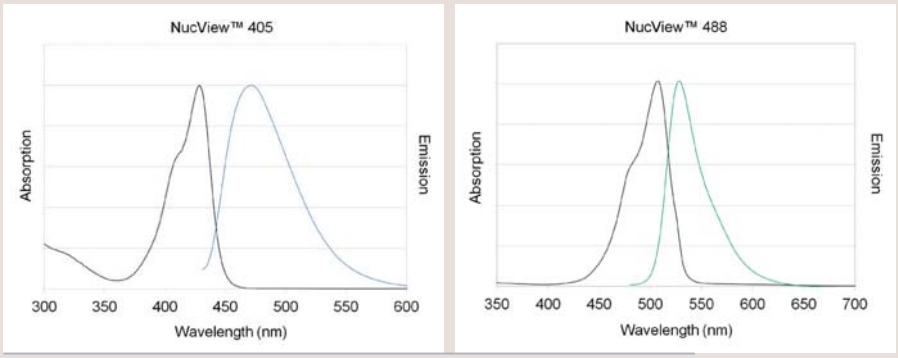


Figure 2.
A. Absorption and emission spectra of blue fluorescent NucView™ 405 dye with DNA.
B. Absorption and emission spectra of green fluorescent NucView™ 488 dye with DNA.

293-H	HaCaT	MDA-MB-231	RINm5F
293-T	HCLE	MDA-MB-468	Saos-2
4T1	HeLa	MDCK	SKBR3
67NR	HepT1	MES-SA	SKLMS1
A172	HMEC	MES-SA/DX	STHdh
A204	HL-60	MG-63	SW684
B16F10	HOS	Min 6	SW872
BeWo	HT-1080	MOLT-3	THP-1
CCL-134	HUH6	N19	TK6
CCL-190	Jurkat	NRK	U2OS
CCRF-CEM	JY	NRK-52E	U251
FU-UR-1	K562	PC-3	U373 MG
GE11	LLC-PK1	PC-9	U937
H358	MCF-7	PC12	WEHI 7.2
H9c2	MCF-10A	RD	

Table 1. Immortalised cell lines tested with NucView™488 in published literature.

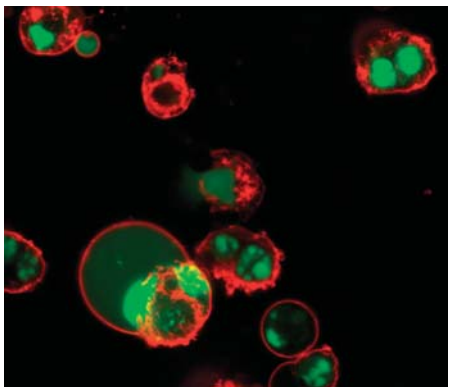


Figure 3. Jurkat cells treated with staurosporine to induce apoptosis, then stained with NucView™ 488 Caspase-3 Substrate and CF™ 594 Annexin V. Apoptotic cells show plasma membrane Annexin V staining (red) and nuclear staining with NucView™ 488 (green).

time detection of apoptosis

Biotium

NucView™ 488 Caspase-3 Substrate has been reported in more than 150 scientific publications in more than 90 different immortalised and primary animal and plant cell types (Table 1 and Table 2).

Green fluorogenic NucView™ 488 Caspase-3 Substrate can be detected by fluorescence microscopy, flow cytometry, or microplate reader in the FITC channel. NucView™ 488 is available in assay kits paired with fluorescent Annexin V conjugates for dual apoptosis marker detection (Figure 3), far-red MitoView™ 633 dye for mitochondrial membrane potential detection (Figure 4), or the far-red dead cell-selective nuclear stain RedDot™2 for simultaneous measurement of apoptosis and necrosis.

Biotium's new blue fluorogenic NucView™ Caspase-3 Substrate is designed for use with the 405 nm laser line for detection by confocal microscopy in the DAPI channel, or by flow cytometry in the Pacific Blue® channel (Figure 5). NucView™405 is ideal for caspase-3 detection in multicolour applications for researchers who wish to reserve the green fluorescence channel for other detection reagents.

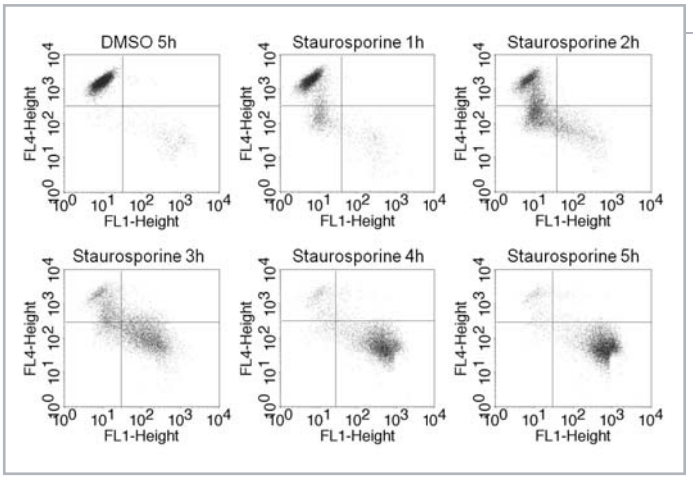
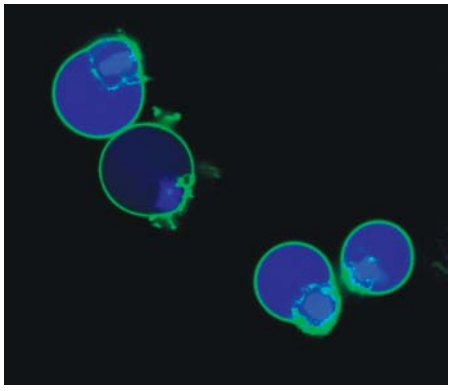


Figure 4. Flow cytometry analysis of caspase-3/7 activity and mitochondrial membrane potential in staurosporine-treated Jurkat cells using the NucView™ 488 and MitoView™ 633 Apoptosis Kit. Green fluorescent NucView™ 488 staining is plotted on FL1 (x-axis) and far-red fluorescent MitoView™ 633 staining is plotted on FL4 (y-axis). As apoptosis progresses, NucView™ 488 signal increases while MitoView™ 633 signal decreases.



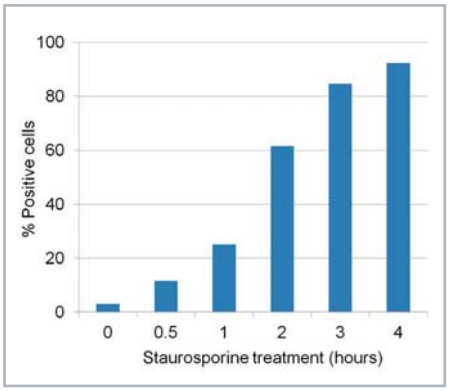


Figure 5. A. Apoptotic Jurkat cell stained with the NucView™ 405 Caspase-3 Substrate (blue) and CF™ 488A Annexin V (green). B. Flow cytometry analysis of apoptosis using NucView™ 405 Caspase-3 Substrate. Jurkat cells were incubated with staurosporine for the times indicated to induce apoptosis, then stained with 1 uM NucView™ 405 caspase-3 substrate for 30 minutes at room temperature. Fluorescence was analysed using a BD LSR II flow cytometer in the Pacific Blue® channel. The graph shows the percentage of NucView™-positive cells at each time point of staurosporine treatment.

Reference:
Cen H., Mao F., Aronchik I., Fuentes R.J., Firestone G.L. (2008). FASEB J 22 (7): 2243-52.

Adipose mesenchymal stem cells (human)	Idiopathic pulmonary fibrosis fibroblasts (human)	Pancreatic beta cells
Alveolar epithelial cells (mouse)	Immature B cells (mouse)	Pancreatic islet cells (mouse)
Cortical neurons (rat)	Kidney epithelial cells (mouse)	Peritoneal macrophages (mouse)
Dendritic cells (mouse)	Lung microvascular endothelial cells (human)	Pollen tubes (field poppy)
Embryonic fibroblast (mouse)	Macrophages (mouse)	Retinal pigmented epithelial cells (human, mouse)
Embryo tailbud (chicken)	Mammary epithelial cells (3-D cultures) (mouse)	Skin fibroblasts (sand cat)
Gingival fibroblasts (human)	Neutrophils (human)	Stem cells (human)
Glia (rat)	SVZ neural progenitor cells	Thymocytes (mouse)
Hemocytes (silkworm)	Oligodendrocytes (mouse)	Umbilical vein endothelial cells (human)
Hepatocytes (rat)	Oocytes (bovine, mouse)	Vascular endothelial cells (rat)
Hippocampal neurons (rat)	Pancreatic acinar cells (mouse)	

Table 2. Primary cell types tested with NucView™488 in published literature.

Description	Size	Cat. No.
NucView™ 488 Caspase-3 Enzyme Substrate, 1 mM in PBS	100 µl	10403
NucView™ 488 Caspase-3 Enzyme Substrate, 1 mM in DMSO	100 µl	10402
NucView™ 405 Caspase-3 Enzyme Substrate, 1 mM in DMSO, Trial Size	100 µl	10405-T
NucView™ 405 Caspase-3 Enzyme Substrate, 1 mM in DMSO	100 µl	10405
NucView™ 488 Caspase-3 Assay Kit for Live Cells, Trial Size	25 assays	30029-T
NucView™ 488 Caspase-3 Assay Kit for Live Cells	100 assays	30029

Description	Size	Cat. No.
Dual Apoptosis Assay with NucView™ 488 and CF™594 Annexin V	50 assays	30067
Dual Apoptosis Assay with NucView™ 488 and CF™640R Annexin V	50 assays	30073
NucView™ 488 and MitoView™ 633 Apoptosis Kit	100 assays	30062
NucView™ 488 and RedDot™2 Apoptosis and Necrosis Kit	100 assays	30072

Quantification of concentrated protein sample solutions using direct UV measurement and the QuantiMate 200 Micro-Volume Cuvette

Direct UV absorbance measurement of samples in aqueous solutions is a well-known method for concentration determinations of nucleic acids and proteins. As sample volumes have decreased, there is an increasing requirement for methods that allow quantification of samples of as little as a few microliters.

The QuantiMate 200 Micro-volume Cuvette is a micro-volume measurement accessory that has been specifically designed for precise and accurate quantification of very small volumes of nucleic acid or protein samples. The QuantiMate 200 Micro-Volume Cuvette has a fixed path length of 0.2mm and a path length accuracy of ±5µm.

Conceived as a flexible and easy to use solution, we demonstrate (using Bovine Serum Albumin, BSA) that the QuantiMate 200 Micro-Volume Cuvette provides highly accurate results for micro-volume measurements when used in combination with a standard UV/Vis spectrophotometer. This enables researchers to perform micro-volume measurements on the same spectrophotometer where they have been using standard (10 mm path length) cuvettes.

QuantiMate 200 cuvette, 0.2 mm path length, ±5 µm accuracy, Z height = 15 mm, PN# 80-7100-71. Second image shows a sample being added to the cuvette for measurement

MATERIALS AND METHODS

QuantiMate Micro-Volume Cuvette
QuantiMate 200 cuvette, 0.2 mm path length, Z height = 15 mm, Cat. No. 730-1451.

UV/Vis spectrophotometer
Biochrom Libra S60 double beam UV/Vis absorbance spectrophotometer, read Z height = 15 mm.

Sample preparation

Lyophilised Bovine Serum Albumin (BSA) from Sigma (Cat. No. A9418) was weighed with a precision scale (d=0.1 mg) and diluted to 40 mg/ml or 270 mg/ml in a PBS buffer pH7 containing 0.01%Brij35. Both master samples were subsequently diluted for constituting new samples. Each sample was homogenised using a vortexer and centrifuge.

Reference readings were taken from three set of samples using Biochrom 30+ Amino Acid Analyser (AAA). The samples were prepared according to the procedure provided in the Biochrom 30+ AAA user manual.

Absorbance measurements and concentration determination

Sample absorbance was measured at two wavelengths: 260 and 280 nm so as to determine the concentration at 280 nm and to check the

variability of the purity ratio at 280/260 nm. The concentration of BSA was calculated by using a concentration factor of 1.386 which is specific to the absorbance of BSA in solution at 280 nm. As this factor is normalised for a standard 10 mm path length, a path length correction factor was also used in the equation. The path length correction factor for the QuantiMate 200 is 50 (10/0.2 mm=50). The resulting calculation was used to determine the concentration of BSA in the protein sample solutions:

[BSA] (mg/ml) = A280 x 1.386 x 50
where A_{280} is the sample absorbance value at 280 nm

The lowest and highest detection limits for BSA were defined based on the Biochrom Libra S60 specification, using a 2 standard deviation confidence interval and the properties of BSA.

Linearity assessment at maximum and minimum concentration ranges

Sample concentrations starting from 1 to 150 mg/ml and from 0.56 to 40 mg/ml were used for determining linearity of the measurements made by the cuvette. The calculated concentrations determined were plotted against the expected BSA concentrations as obtained from the serial dilution factors, the master solution concentrations and the measurements obtained from the Biochrom 30+ Amino Acid Analyser.

Sample solution measurement accuracy, precision and carryover

Three different sample concentrations (40, 20 and 10 mg/ml) were measured repeatedly to determine accuracy, precision and sample carryover. Each absorbance was read 10 times consecutively using 3 µl of sample in the cuvette. In each sample series of a specific concentration, measurements of a blank were performed. Each sample was removed with a lint-free tissue before taking a new measurement. The resulting measured concentration was plotted against the sample type and replication number.

RESULTS AND DISCUSSION

Determination of the dynamic protein detection range using the QuantiMate 200 Micro-Volume Cuvette and the Biochrom Libra S60 UV/Vis spectrophotometer

The spectrophotometer detection limit which defines the minimum and maximum concentration that can be used for a given sample type; therefore, it was important to understand how the QuantiMate Micro-Volume Cuvette would influence the sample detection range when used with a defined spectrophotometer. Based on the noise specifications of the Libra S60 UV/Vis spectrophotometer, the calculated lower limit of detection (LOD) corresponded to 0.5 mg/ml for BSA. While the lower detection range would be easily explained by the spectrophotometer, the highest detection limit would be determined by BSA whose physicochemical properties would change significantly at increased concentrations, close to 150 mg/ml (Milton 2007).

The results (Figure1 and Figure 2) showed an excellent correlation between the expected and measured BSA concentration at values ranging from 0.5 to 150 mg/ml, which matched the previously defined dynamic detection range.

This data indicates that the QuantiMate 200 Micro-Volume Cuvette is capable of measuring a broad dynamic range using only a single path length. This can lead to a significant improvement of the quality of the results obtained with microvolume accessories where multiple path lengths are often required to achieve a similarly broad dynamic range, often resulting in calculation errors.

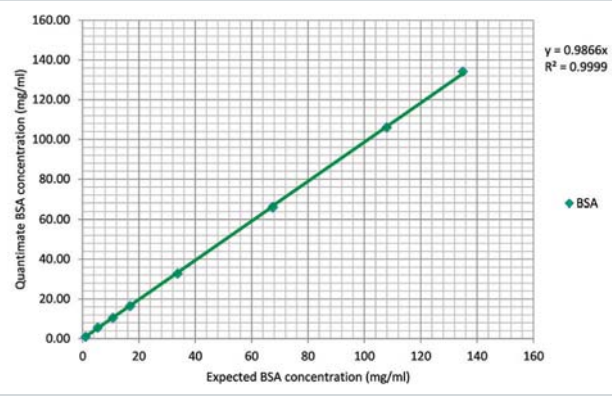


Figure 1. BSA concentrations ranging from 130 to 1 mg/ml using QuantiMate 200 and Biochrom Libra S60, plotted against measured values. Samples were obtained from the 270 mg/ml master solution which was diluted in series. The absorbance was measured using the QuantiMate 200 Micro-Volume Cuvette and the Biochrom Libra S60 UV/Vis spectrophotometer. The absorbance at 280 nm was used to calculate the concentration (QuantiMate BSA Concentration). The expected concentrations were obtained from the weight of the BSA and AAA analysis. The fit was defined via linear regression method, forced through the origin.

Figure 2. BSA concentrations plotted against measured values using the QuantiMate 200 Micro-Volume Cuvette and Biochrom Libra S60 spectrophotometer. Samples were obtained from the 40 mg/ml master solution which was diluted via serial dilutions. The absorbance was read with QuantiMate 200 Micro-Volume Cuvette and the Biochrom Libra S60 UV/Vis spectrophotometer. 280 nm was used for concentration calculation (QuantiMate concentration). The measured concentrations were obtained from weighed BSA and AAA analysis. The fit was defined using linear regression method forced through the origin.

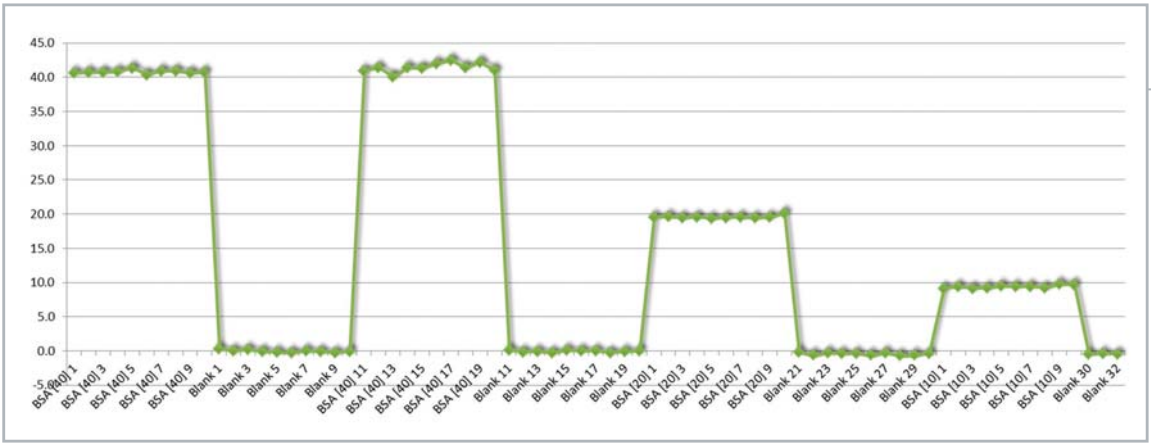


Figure 3. Protein sample carryover determination. 40, 20 and 10 mg/ml of BSA separated with a blank are displayed as examples. The expected protein concentrations, in mg/ml are indicated in brackets, next to the sample name, followed by the replication number.

Protein accuracy and precision measurements using the QuantiMate 200 Micro-Volume Cuvette and the Biochrom Libra S60 UV/Vis spectrophotometer

The accuracy and precision of measurements are critical parameters for understanding the reliability of sample concentration determinations. The Biochrom 30+ Amino Acid Analyser is a standard for very accurate protein quantification and was used to assess the accuracy of the measurements taken with the QuantiMate 200 Micro-Volume Cuvette.

Table 1. Determination of protein measurement accuracy and precision with the QuantiMate 200 Micro-Volume Cuvette and Biochrom Libra S60 UV/Vis spectrophotometer.

BSA concentrations (mg/ml)		
Measured	QuantiMate 200	%CV
41.32	40.75	0.62%
19.74	19.53	1.01%
9.88	9.36	1.66%

Measured: AAA results based on phe, arg and tyr in BSA, Read: QuantiMate 200 Micro-Volume Cuvette and Libra S60 UV/Vis spectrophotometer.
SD: Standard deviation, CV(%): coefficient of variation for QuantiMate results.

The results shown in Table I indicate that the readings were highly accurate with low variability. It is well known that protein solutions are often heterogeneous as viscosity varies with concentration which increases turbidity. The resulting variability was reduced in the BSA samples by centrifugation prior to sample measurement (Aitken and Learmonth 2009).

The concentration of protein samples was shown to be determined very accurately and precisely using the QuantiMate 200 Micro-Volume Cuvette in a Biochrom Libra S60 UV/Vis spectrophotometer.

Protein sample carryover using the QuantiMate 200 Micro-Volume Cuvette and the Biochrom Libra S60 UV/Vis spectrophotometer

The ability to repeatedly and quickly measure accurate concentrations is crucial if you need to rely on the results for an important downstream experiment. Using an appropriate method for removing sample and cleaning the cuvette enables the easy transfer of one sample solution to the next. The QuantiMate Cleaning Guide recommends an easy one step process consisting of wiping off the sample reservoir using a lint-free tissue before adding a new sample.

Repeated measurements of protein followed by blank measurements demonstrated that each subsequent sample and blank measurements gave the expected concentrations values (Figure 3). The blank data set showed no concentration shift from the sample measurements that would be attributable to protein contamination, indicating that sample carryover was undetectable at each of the tested concentrations.

The design of the QuantiMate Micro-Volume Cuvette allowed uncomplicated sample removal and cleaning which is necessary in between sample measurements; this cleaning procedure was found to not affect the consistency or the reliability of the data.

CONCLUSION

QuantiMate Micro-Volume Cuvette delivers dynamic detection range and accuracy

QuantiMate 200 Micro-Volume Cuvette displayed a broad dynamic detection range which was both precise and accurate. In addition, the QuantiMate Micro-Volume Cuvette has a path length accuracy of ±5 µm reducing error over the platform allowing the QuantiMate Micro-Volume Cuvette to achieve a high level of accuracy.

QuantiMate Micro-Volume cuvette offers ease of use

The QuantiMate Micro-Volume Cuvette is very easy to use. No specific sample preparation is required and the loading and removal of sample solution from the cuvette is straightforward and quick. As a result the QuantiMate Micro-Volume Cuvette is an appropriate choice for busy laboratories requiring the determination of broad protein concentration ranges using a single microvolume device and a reliable spectrophotometer.

QuantiMate Micro-Volume Cuvette provides versatility and affordability

Based on the earlier observations, broad concentrations of other proteins can be determined accurately using a reliable spectrophotometer.

Compared to other more complex quantification techniques, QuantiMate is a reasonably priced and reliable alternative for quick quantification of concentrated protein samples in aqueous solutions.

Reference
- *Aitken, A., and M.P., Learmonth. 2009. Protein determination by UV absorption. In The Protein Protocols Handbook. Third Edition. J.M. Walker, editor. Humana Press, New York, pp. 3-6.*
- *Minton, Allen P. 2007, Static light scattering from concentrated protein solutions, I: general theory for protein mixtures and application to self-associating proteins. Biophys. J. 93: 1321–1328*

BTX® HARVARD APPARATUS
The Electroporation Experts

Disruption of blood brain barrier using electroporation

Hjouj M. et al., MRI Study on Reversible and Irreversible
Electroporation Induced Blood Brain Barrier Disruption 2012, PLoS One.

ABSTRACT

Electroporation, is known to induce cell membrane permeabilisation in the reversible (RE) mode and cell death in the irreversible (IRE) mode. Using an experimental system designed to produce a continuum of IRE followed by RE around a single electrode we used MRI to study the effects of electroporation on the brain. Fifty-four rats were injected with Gd-DOTA and treated with a G25 electrode implanted 5.5 mm deep into the striata. MRI was acquired immediately after treatment, 10 min, 20 min, 30 min and up to three weeks following the treatment using: T1W, T2W, Gradient Echo (GE), serial SPGR (DCE-MRI) with flip angles ranging over 5 – 25°, and diffusion-weighted MRI (DWMRI). Blood brain barrier (BBB) disruption was depicted as clear enhancement on T1W images. The average signal intensity in the regions of T1-enhancement, representing BBB disruption, increased from 1887 ±83 (arbitrary units) immediately post treatment to 2246 ±94 20 min post treatment, then reached a plateau towards the 30 min scan where it reached 2289 ±87. DWMRI at 30 min showed no significant effects. Early treatment effects and late irreversible damage were clearly depicted on T2W. The enhancing volume on T2W has increased by an average of 2.27 ±0.27 in the first 24 – 48 hours post treatment, suggesting an inflammatory tissue response. The permanent tissue damage, depicted as an enhancing region on T2W, 3 weeks post treatment, decreased to an average of 50 ±10% of the T2W enhancing volumes on the day of the treatment which was 33 ±5% of the BBB disruption volume. Permanent tissue damage was significantly smaller than the volume of BBB disruption, suggesting, that BBB disruption is associated with RE while tissue damage with IRE. These results demonstrate the feasibility of applying reversible and irreversible electroporation for transient BBB disruption or permanent damage, respectively, and applying MRI for planning/monitoring disruption volume/shape by optimising electrode positions and treatment parameters.

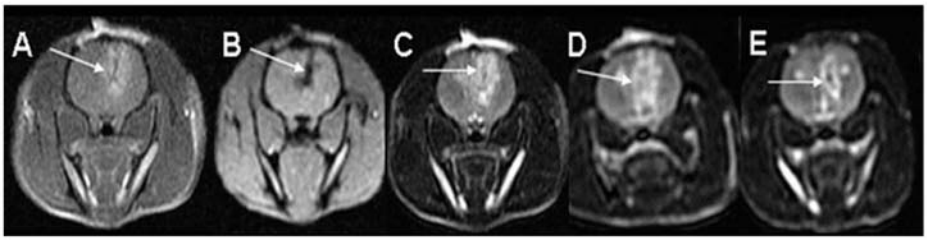
METHODS

Intracranial electrode placement

The bregma was identified through a midline scalp incision, and one 1 mm burr hole was drilled in the right or left region of the skull, 3 mm anterior and 2 mm lateral to the bregma. 25 gauge stainless steel electrodes were placed stereotactically in the striatum at a depth of 5.5 mm. A second, large 4 cm by 8 cm flat electrode was pressed against the rat chest after applying conducting gel for better electric coupling. The electrodes were connected to the pulse generator. Control rats Underwent similar procedures, including electrode implantation, without applying the electric pulses.

Electroporation treatment protocol

Rats were treated using a conventional electroporator power supply (BTX 830 or Gemini X2; Harvard Apparatus, Holliston, MA). Voltages used in the experiments ranged from 250 to 650 V, the number of pulses ranged from 50 to 90, the pulse duration ranged from 50 to 70 ms, and the pulse delivery frequency was 4 Hz.



MRI sequences for depicting electroporation effects in the rat brain

T1W (A), Gradient-Echo (B) and T2W (C–E) MRI of a rat treated with one intracranial electrode and another external flat electrode pressed against the rat chest. Treatment was performed with 50 pulses of 650 V, 70 µs duration and a frequency of 4 Hz. Significant BBB disruption is depicted as bright enhancement on the T1W images acquired 30 min after treatment (A). The GE image (B) shows signal void along the path of the electrode suggesting haemorrhage. T2W images depict tissue response to the treatment as bright enhancement (C–E). It can be seen that 1 day post treatment (D) the volume of tissue changes seems larger than on the day of the treatment (A), but then the volume is reduced by day 8 (E)

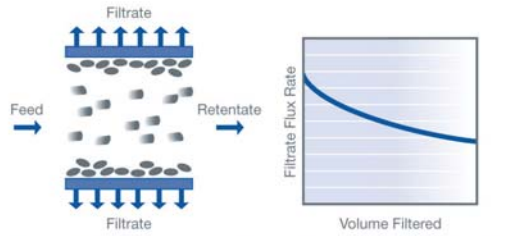
RESULTS

Application of thermal reversible electroporation and non thermal irreversible electroporation electric fields to the rat brain demonstrates the feasibility of applying electroporation for significant and transient BBB disruption with and without permanent tissue damage, under real time MR treatment monitoring and with late MR monitoring of treatment effects. Significant correlation was found between treatment voltage extent of NTRE and BBB disruption volume. BBB disruption volume was significantly correlated with later volume of tissue damage and in all cases depicted a larger volume than the final damage. These results imply that MRI may be used for treatment monitoring of brain electroporation where preservation of healthy tissue is crucial. Furthermore, electroporation may be applied for a combined treatment of systemic chemo + local electroporation, for destruction of brain tumour tissue by IRE, while chemotherapy is efficiently delivered to the surrounding infiltrated tissue due to the larger coverage of temporary BBB disruption.

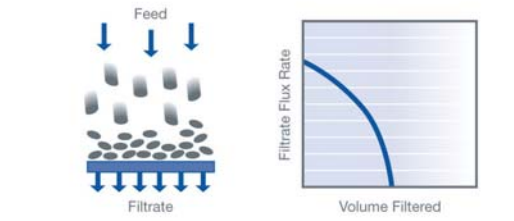
Increased productivity using Pall's Minimate™ Capsules

Ultrafiltration (UF) techniques rely on the use of fluid pressure to drive the migration of the smaller molecules through a UF membrane with the simultaneous retention of larger molecules. While chemical precipitation can be used to concentrate a protein sample, separation with ultrafiltration is based on mechanical rather than chemical interactions allowing a researcher to perform sample concentration without the addition of denaturing solvents or salts. Buffer exchange using dialysis technologies uses large volumes of buffer and, since the only force acting upon the solution is diffusion, the process can take several days. Pre-assembled and simple to use ultrafiltration devices can rapidly perform either concentration or buffer exchange procedures without the extensive handling required for many other techniques.

Figure 1.



(A) Sample solution flows through the feed channel and along (tangent to) the surface of the membrane as well as through the membrane. The crossflow prevents build up of molecules at the surface that can cause fouling. (B) The TFF process prevents the rapid decline in flux rate seen in direct flow filtration allowing a greater volume to be processed per unit area of membrane surface.



(A) The feed is directed into the membrane. Molecules larger than the pores accumulate at the membrane surface to form a gel, which fouls the surface, blocking the flow of liquid through the membrane. (B) As the volume filtered increases, fouling increases and the flux rate decreases rapidly.

Optimisation of ultrafiltration using cross flow

Ultrafiltration can be performed in one of two operational modes: Direct Flow Filtration (DFF), or Tangential Flow Filtration (TFF) (Figure 1). DFF works well for small volumes (up to 60 ml) using centrifugal devices, however, DFF can fall prey to problems with membrane fouling. To reduce the formation of a gel layer, cross flow can be generated on the upstream side of the membrane using a floating stir bar configuration (stirred cell) or by creating a controlled laminar flow. While stirred cell operations tend to improve UF performance, they are still limited in achieving the optimal performance since the velocity and subsequent level of agitation is dependent on the sweep of the bar that varies along the radius of the sweep.

Tangential flow filtration (TFF), also known as cross flow filtration, is a rapid and efficient method for separation and purification of biomolecules. TFF can be used to concentrate and desalt sample solutions ranging in volume from 10 ml to thousands of litres. It can be used to fractionate large from small biomolecules, harvest cell suspensions and clarify fermentation broths and cell lysates.

During the TFF process the feed stream passes parallel to the membrane face as one portion passes through the membrane

Diagram 1.

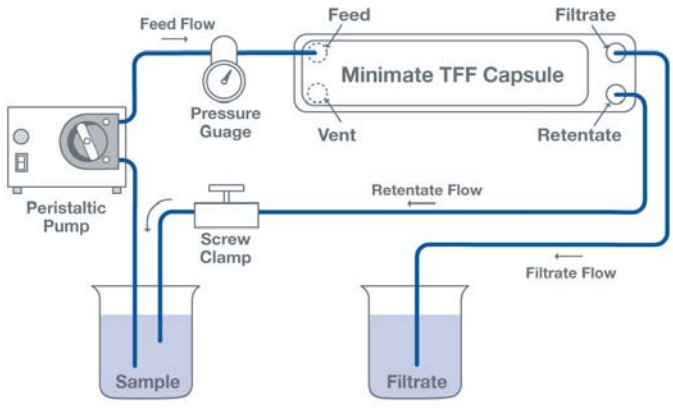


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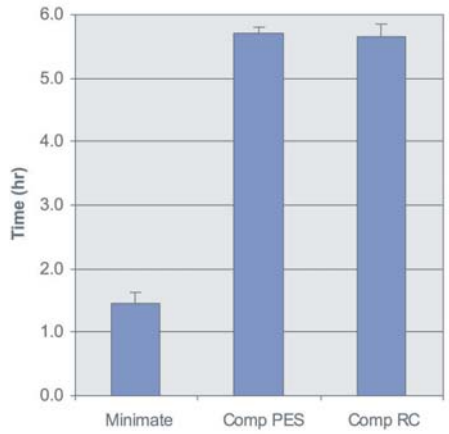
Figure 2.



A 2 mg/mL BSA solution was concentrated ten-fold (1000 to 100 mL) in either a 350 mL stirred cell device or Minimate capsule. The Minimate contains a pre-assembled Omega 10K membrane. The crossflow, set at 50 mL/min with retentate loop backpressure applied to create an initial filtrate flow of about 15 mL/min. The stirred cell devices used polyethersulfone (PES) or regenerated cellulose (RC) disks and were pressurized with filtered air at 55 psi giving a starting filtrate flow of about 6 mL/min. Error bars indicate standard error for five independent runs.

Minimate™ TFF system	
Description	**Cat. No.**
Minimate™ TFF system, EU-plug	516-0186
Minimate™ TFF system, UK-plug	516-0205
Minimate™ TFF Capsules with Omega™ Membrane	
MWCO (kD)	
3	515-0117
5	515-0118
10	516-6879
30	516-6880
50	515-0119
70	515-0120
100	516-6881
300	515-0121
500	515-0122
1000	515-0123

(permeate) while the remainder (retentate) is recirculated back to the feed reservoir. TFF can be performed in a simple system, for example, Pall's Minimate™ system (see Diagram 1).

Reduce processing time using the Minimate™ Capsule

TFF operations allow a uniform and gentle recirculation of sample flow over the surface of a membrane effectively controlling membrane fouling. Use of the entire membrane surface results in improved flux rates significantly reducing processing times and increasing productivity. The Minimate™ Capsule is pre-assembled, ready to use and can process volumes up to 1 litre without the need for user intervention. In contrast, most stirred-cell devices have a 350 ml or smaller processing capacity and must be opened repeatedly, risking integrity failures and requiring valuable technician time.

Processing time is a function of overall device performance. The processing operation of five Minimate™ 10K omega capsules was directly compared to similar processing conditions using comparable stirred cell configurations containing high flow PES or regenerated cellulose membranes. A 2 mg/ml BSA solution (1 litre) was concentrated ten-fold (to 100 ml) using the Minimate™ Capsule to achieve concentration in just over an hour (Figure 2). In contrast, processing times for stirred cell devices took five times longer regardless of membrane configuration and required the user to refill the device repeatedly.

Pall's Minimate™ system is designed to work with the Minimate™ TFF Capsule, this easy to use system can process sample volumes up to 1 l or more and efficiently concentrate samples to as little as 5 ml. Subsequent desalting or buffer exchange steps can be run on the same system with minimal user intervention.

- Ready to use – plug in a Minimate™ TFF Capsule, add sample and turn on the pump to start processing. The Minimate™ TFF system includes all the hardware, tubing and fittings needed to get your TFF process up and running quickly
- High concentration factors – the low system working volume achieved through the use of a conical bottom reservoir and compact design enable high concentration factors from up to 1 l or more of sample to be achieved. Concentrate your sample down to as little as 5 ml
- Efficient – continuous diafiltration for desalting or buffer exchange can be performed unattended. The diafiltration buffer container is connected to the lid of the feed reservoir
- Minimate™ TFF Capsules contain a robust, polyethersulphone, ultrafiltration (UF) membrane. The Omega™ UF membrane offers low non specific adsorption characteristics resulting in high product recovery. Minimate™ TFF capsules are available with a wide range of molecular weight cut-offs

A protein estimation standard, comparable to BSA, without public safety & regulatory issues

The traditional method for calculating unknown protein concentrations is to use a standard curve generated from known protein standards.

The most reliable protein estimation is performed using a reference or a protein standard that has properties similar to the protein being estimated. Often, it is difficult to find a protein standard with similar properties to the sample being analysed. As a result, it has become acceptable to use readily available proteins such as bovine serum albumin (BSA) and gamma globulin as standards.

BSA is by far the most popular protein standard on the market routinely packed with commercial protein estimation kits. Unfortunately, the emergence of bovine spongiform encephalopathy (BSE) (mad cow disease) in the late 1980s had led to ever tightening regulatory controls on import and export of animal products, particularly bovine. This has resulted in increased pricing or even inability to purchase kits and products with BSA.

To overcome these regulatory issues and offer protein estimations kits and standards, G-Biosciences have developed a non animal protein standard that shows a colorimetric response similar to BSA in multiple protein assays. This makes G-Biosciences' Non-Animal Protein Standard a suitable replacement for BSA allowing for circumvention of regulatory issues.

AIM

To evaluate the Non-Animal Protein Standard by comparing to BSA in the following protein assays:

- **BCA**
- **Lowry**
- **NI™ (Non-Interfering™)**
- **Bradford**
- **CB-X™**
- **RED660™**

METHOD

Preparation of protein standards
2 mg/ml of BSA Protein Standard (Cat. No. 786-006) or Non-Animal Protein Standard (Cat. No. 786-438) were serially diluted with 1X phosphate buffered saline to give final concentrations of 0.0625, 0.125, 0.25, 0.5 and 1 mg/ml. 50 µl of each dilution was used in each protein estimation assay.

BCA protein assay (Cat. No. 786-571)
50 parts of BCA solution were combined with 1 part copper solution and 1 ml of this working solution was added to the protein standards. The assay was incubated at 37 °C for 30 minutes and then read at 562 nm.

Lowry protein assay (Cat. No. BE-402L)
100 µl copper solution was added to each standard and mixed by vortexing. 1 ml Folin Ciocalteu reagent was then added and the reaction incubated at room temperature for 30 minutes. The assay was read at 650 nm.

NI™ (Non-Interfering™) protein assay (Cat. No. 786-005)
This assay is a copper ion based assay, similar to BCA and Lowry protein assays, but has additional clean up steps for improved accuracy.

500 µl Universal Protein Precipitating Agent I (UPPA™) was added to each protein standard and incubated for 3 minutes at room temperature. Next 500 µl UPPA™ II

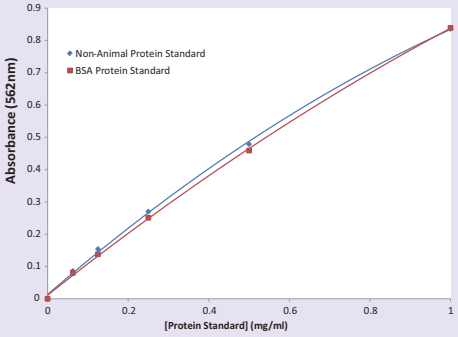


Figure 1. Comparison of BSA and Non-Animal Protein Standard with the BCA protein assay.

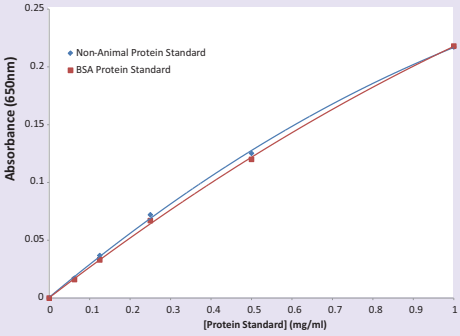


Figure 2. Comparison of BSA and Non-Animal Protein Standard with the Lowry protein assay.

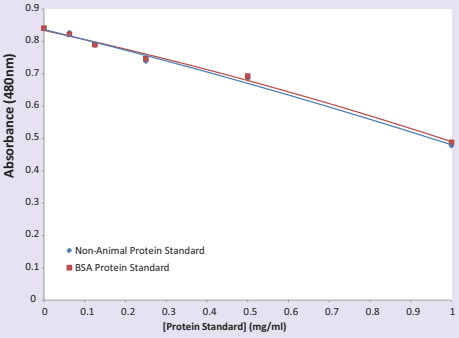


Figure 3. Comparison of BSA and Non-Animal Protein Standard with the NI™ protein assay.

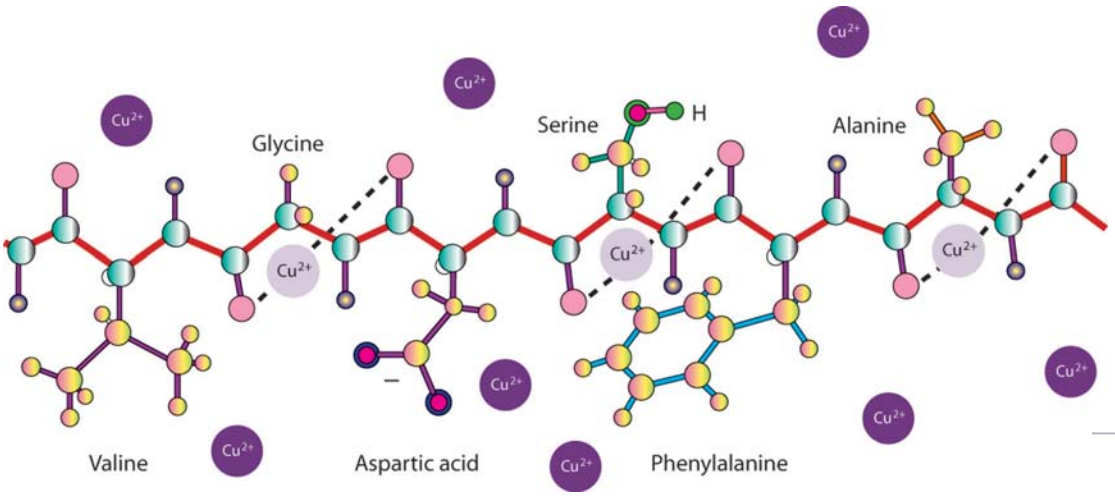


Figure 4. The interaction of copper ions with proteins.

was added and mixed by vortexing. The protein was collected by centrifugation at 16 000 xg for 5 minutes. The supernatant was removed and 100 µl copper solution and 400 µl deionised water were added. Following vortexing to dissolve the protein pellet, 1 ml Reagent II was added. The assay was incubated for 20 minutes at room temperature and then read at 480 nm.

Bradford protein assay (CB™ Protein Assay, Cat. No. 786-012)
1 ml Bradford (CB™ Protein Assay Reagent) was added to each standard and then incubated at room temperature for 5 minutes. The absorbance was read at 595 nm.

CB-X™ protein assay (Cat. No. 786-11X)
This assay is an enhanced dye binding protein assay, similar to a Bradford protein assays, but has additional clean up steps for improved accuracy.

1 ml CB-X™ reagent was added to the standards and mixed by vortexing. The precipitated protein was collected by centrifugation at 16 000 xg for 5 minutes and the supernatant was discarded. 50 µl CB-X™ Solubilization Buffer-I and 50 µl CB-X™ Solubilization Buffer-II were added to the pellet and then vortexed to dissolve the protein pellet. 1 ml CB-X™ Assay Dye was added and incubated at room temperature for 5 minutes. The absorbance was read at 595 nm.

RED660™ protein assay (Cat. No. 786-676)
1 ml RED660™ Protein Assay Reagent was added to each protein standard and mixed. The assay was incubated for 5 minutes at room temperature and the absorbance read at 660 nm.

RESULTS

Figures 1 - 3 show a comparison between BSA and the Non-Animal Protein Standard using copper ion-based assays (Figure 4). In the copper ion-based protein assays, the protein solution is mixed with an alkaline solution of copper salt. Under alkaline conditions, cupric ions (Cu^{2+}) chelate with the peptide bonds resulting in reduction of cupric (Cu^{2+}) to cuprous ions (Cu^{+}). If the alkaline copper is in excess over the amount of peptide bonds, some of the cupric ions (Cu^{2+}) will remain unbound to the peptide bonds and are available for detection. Protein assays based on copper ions can be divided into two groups, assays that detect reduced cuprous ions (Cu^{+}) and that detect unbound cupric (Cu^{2+}) ions.

The cuprous ions are detected either with bicinchoninic acid (BCA) (Figure 1) or Lowry assay's Folin Reagent (phosphomolybdic/phosphotungstic acid) (Figure 2).

The NI™ protein assay (Figure 3) is based on the detection of unbound cupric ions; the protein solution is mixed with an amount of alkaline copper that is in excess over the amount of peptide bond. The unchelated cupric ions are detected with a colour-producing reagent that reacts with cupric ions. The amount of colour produced is inversely proportional to the amount of peptide bond.

Figures 5 - 7 show a comparison between BSA and the Non-Animal Protein Standard using dye binding assays. The dye binding protein assays are normally based on the binding of protein molecules to Coomassie dye under acidic conditions. The binding of protein to the dye results in spectral shift, the colour shifts from brown (A^{max} = 465 nm) to blue (A^{max} = 610 nm). The change in colour density is read at 595 nm and is proportional to protein concentration. The basic amino acids, arginine, lysine and

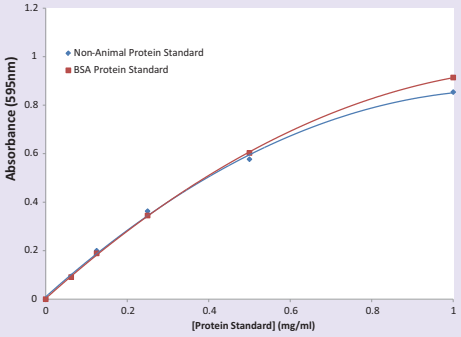


Figure 5. Comparison of BSA and Non-Animal Protein Standard with the Bradford protein assay.

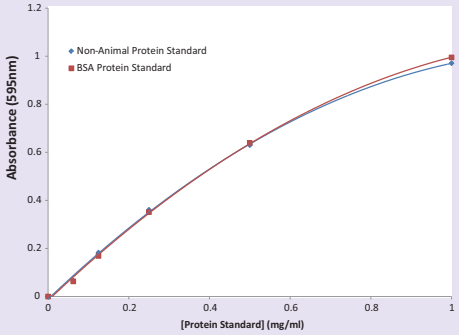


Figure 6. Comparison of BSA and Non-Animal Protein Standard with the CB-X™ protein assay.

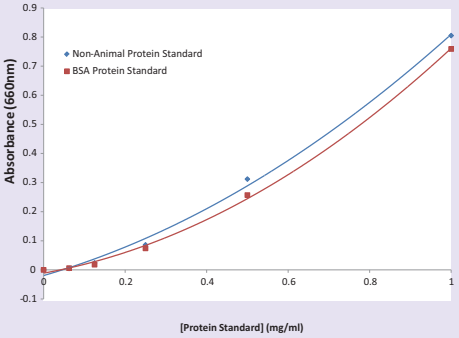


Figure 7. Comparison of BSA and Non-Animal Protein Standard with the RED660™ protein assay.

histidine play a role in the formation of dye-protein complexes colour.

The Bradford Protein Assay (Figure 5) and the CB-X™ Protein Assay (Figure 6) are Coomassie dye based assays.

RED660™ protein assay (Figure 7) is a dye binding assay using a proprietary dye that has a colour shift to 660 nm.

DISCUSSION

G-Biosciences' Non-Animal Protein Standard shows colorimetric responses similar to the commonly used bovine serum albumin standards in both copper ion-based assays and dye binding protein assays.

This similarity makes the Non-Animal Protein Standard an excellent alternative to BSA allowing it to be directly substituted into a large selection of protein assays.

An additional advantage is that G-Biosciences' Non-Animal Protein Standard does not require any special regulatory controls, import or excise taxes or duties.

Description	Size	Cat. No.
Non-Animal Protein Standard (pre-diluted, [0.1 – 1 mg/ml])	6 x 5ml	786-439
Non-Animal Protein Standard [2 mg/ml]	2 x 5ml	786-438
Bovine Serum Albumin Standard (pre-diluted [0.1 - 1.0 mg/ml])	6 x 5ml	786-114
Bovine Serum Albumin Standard [2 mg/ml]	2 x 5ml	786-006
Bicinchoninic Acid (BCA) protein assay	500x 1 ml assays 2500x microwell assays	786-570
Bicinchoninic Acid (BCA) protein assay	1000x 1 ml assays 5000x microwell assays	786-571
Bicinchoninic Acid (BCA) protein assay with Non-Animal Protein Standard	500 assays/ 2500 micro-assays	786-890
Bicinchoninic Acid (BCA) protein assay with Non-Animal Protein Standard	1000 assays/ 5000 micro-assays	786-891
Micro Bicinchoninic Acid (BCA) protein assay	500x 1 ml assays >3300x microwell assays	786-572
Micro Bicinchoninic Acid (BCA) protein assay with Non-Animal Protein Standard	500 assays/ 3300 micro-assays	786-895
Bicinchoninic Acid (BCA) reducing agent compatible protein assay	250x 1 ml assays	786-573
Bicinchoninic Acid (BCA) protein assay: Reducing agent compatible with Non-Animal Protein Standard	250x 1 ml assays	786-892
NI™ (Non-Interfering™) Protein Assay Kit with albumin standard	500 assays	786-005
NI™ (Non-Interfering™) Protein Assay Kit with Non-Animal Protein Standard	500 assays	786-896
CB™ protein assay trial	15 assays	786-012T
CB™ protein assay with albumin standard	500 assays	786-012
CB™ protein assay with Non-Animal Protein Standard	500 assays	786-893
CB-X™ protein assay	10 assays	786-12XT
CB-X™ protein assay with Non-Animal Protein Standard	500 assays	786-894
CB-X™ protein assay (no BSA standard)	500 assays	786-11X
CB-X™ protein assay with albumin standard	500 assays	786-12X
RED 660™ protein assay	500 assays/ 2500 micro-assays	786-676
RED 660™ protein assay with Non-Animal Protein Standard	500 assays/ 2500 micro-assays	786-899

Inside the Biotix Robotic Laboratory

With the continuing trend of labs incorporating liquid handling automation into their workflows, performance and quality are top considerations when selecting the right pipette tips to use with these systems. For high throughput automation, robotic tips are expected to meet stringent quality manufacturing standards. QC processes must ensure batch-to-batch conformity as well as meet exacting requirements for straightness, sealing, purity, accuracy and precision.

In order to manufacture robotic tips to the highest engineering standards for automation systems, Biotix has built an in-house Robotic Laboratory as a key element of its QC process for validation and release testing. Unlike other manufacturers that only perform dimensional inspection to engineering drawings, Biotix requires both dimensional and functional testing. A combination of specialised fixtures and functional application tests are used, and executed using specific automated liquid handling system that the tips have been designed for. Experienced technicians run the products through a world class quality system measuring coefficient of variance (CV), total indicated run out (TIR), insertion force and many other performance tests on the robots. If a product fails any test it is rejected, ensuring that only the highest quality tips reach the end user's lab.

As a result, Biotix ultra-straight tips target the centre of microplate wells precisely, delivering exceptional accuracy and precision without risk of jamming, offering the highest quality robotic tips the industry has ever seen with specifications to ensure complete compatibility. Use of the standard definitions bundled in the robotic software programme is appropriate when using Biotix robotic tips. These tips are also BioReady™ certified to be free of RNase, DNase and Endotoxin (pyrogens).

Biotix robotic tips are compatible with these popular brands of automated liquid handling systems:

Agilent®
Beckman®
Tecan®
Caliper®
BioTek®
PerkinElmer/Packard®
Molecular Devices®

For more information on Biotix robotic tips contact your local VWR representative or visit vwr.com

Biotix™
Fluid Innovation

Highlighting innovative design features and useful application information for:

Thermo Scientific manual liquid handling

smart notes

Design and innovation ▶ Adjustable tip spacing

Q

Is there an easier and more efficient way to transfer liquids between various labware formats, than using traditional handheld pipettes?

A

Yes. The adjustable tip spacing feature on the Thermo Scientific™ E1-ClipTip™ Electronic Equalizer multichannel pipette allows you to set the distance between pipette tips for different types of labware by simply sliding a handle. This exponentially reduces the number of repetitions and time needed to perform many common applications.

From test tubes to deepwell blocks; microcentrifuge tubes to 96-well plates. Even 48-well microplates to agarose gels — daily pipetting can require multiple labware formats in an application. Depending on the application a single channel pipette may be the only option when working between multiple labware formats. This dramatically increases the number of repetitive motions, and overall time needed to complete these tasks. The E1-ClipTip Electronic Equalizer multichannel pipettes combine electronic pipetting action with multidispensing mode and adjustable tip spacing to significantly reduce pipetting time. The ClipTip interlocking tip interface ensures that the tips are securely locked and sealed on the pipette, decreasing the risk of uneven sample loading or loose tips to waste valuable samples and time.

What could once only be accomplished through repetitive motions using a single channel pipette, can now be quickly and efficiently performed by using multichannel electronic pipettes. Multidispensing mode, found only in electronic pipettes, enables dispensing of the same volume repetitively with one aspiration.

For instance, aspirate 300 µl of sample and dispense 30 µl, 10 times. The adjustable tip spacing enables you to transfer multiple samples between various labware formats at once with only one movement.

Why?

Adjustable tip spacing can reduce pipetting time by up to 90%

Thermo SCIENTIFIC

Figure 1.

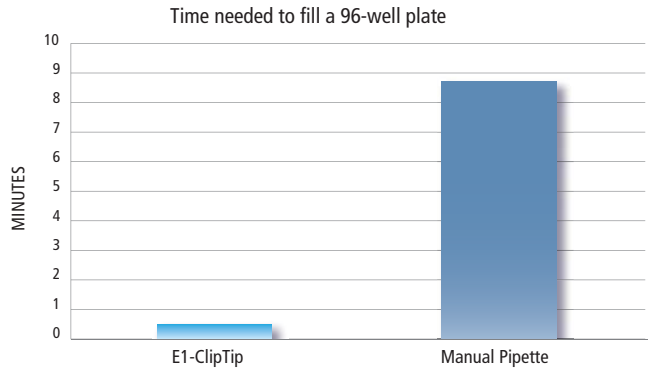


Figure 1. Sample transfer from microcentrifuge tubes to a 96-well microplate.

Using a single channel manual pipette and an E1-ClipTip electronic Equalizer 8-channel pipette, samples were transferred from 8 microcentrifuge tubes to a 96-well microplate. The length of time needed to fill the entire microplate was recorded. Completing the task by using the E1-ClipTip took 90% less time when compared to a single channel manual pipette.

Reduced repetition and strain

A notable part of the time savings is a result of the reduction in motions needed to transfer the liquid from labware to labware. When using an electronic pipette with adjustable tip spacing and multidispensing mode, the repetition of forearm movements can be substantially reduced. In addition, exhausting thumb movements are eliminated by index finger trigger operation and electronic tip ejection. Pipetting becomes more efficient and comfortable decreasing the risk of Repetitive Strain Injury (RSI and lessening the risk of mistakes.

Figure 2.

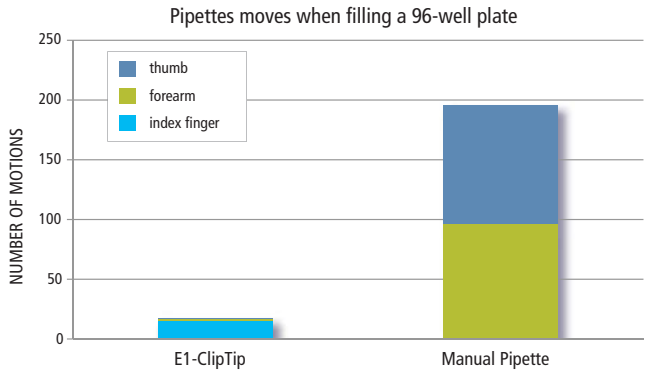


Figure 2. The number of forearm and finger motions required to transfer samples from microcentrifuge tubes to fill up a 96-well microplate.

The number of forearm and finger motions was counted when samples were transferred from 8 separate microcentrifuge tubes to fill up a 96-well microplate. The same pipettes were in use as in the experiment above (Figure 1). With the E1-ClipTip pipette thumb was used only for electronic tip ejection. Using the E1-ClipTip required over 90% less motions when compared to use of a manual single channel pipette.

Summary

When transferring samples between different labware formats, the adjustable tip spacing found on E1-ClipTip Equalizer multichannel electronic pipettes will take up to 90% less time, and reduce 90% of tiresome repetitions compared to single channel pipettes. The reduction in repetitions can lessen the risk of pipetting mistakes and Repetitive Strain Injury (RSI).

See how interlocking ClipTip technology secures tips onto the pipette for reproducible results. Learn more at www.thermoscientific.com/cliptip

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