VWR® We Enable Science

Volume 2, Issue 1
April 2016

VWR bioMarke™

IgGy Antibody Selector

Over **300,000** Antibodies | **30** Suppliers | **1** Source

ABGENT envision proteomics

Abnova

BioLegend The path to legendary discovery™

Bioss ANTIBODIES

Biotium

BioVision Life Science SOURCE™

Diagnostic BioSystems

EMD MILLIPORE

Enzo

Distributor GE Healthcare

GeneTex

GenScript Corporation VWR Strategic Partner

ImmunoReagents Inc.

JACKSON

KPL

MP Biomedicals

Promega

ProSci Incorporated

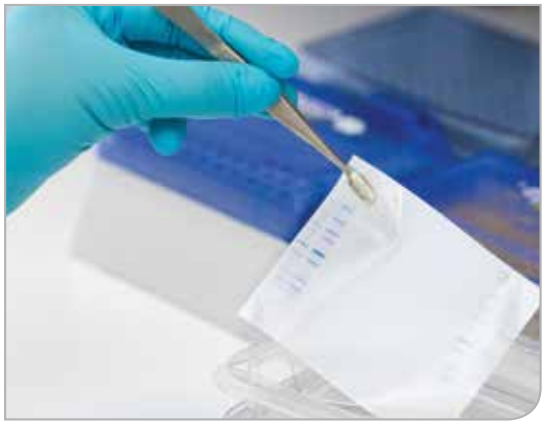
PEPROTECH Manufacturer of Quality Cytokine Products

ROCKLAND antibodies & assays

Thermo SCIENTIFIC

TONBO biosciences Equivalent Performance, Exceptional Value

TREVIGEN®



CONTENTS

Rapid Western Blotting for Streamlined Results

Jennifer Bayraktar, PhD

Western blotting is frequently performed in molecular and cellular biology labs to identify the presence, size, and relative quantity of specific a protein within complex protein mixtures. Proteins are first separated by polyacrylamide gel electrophoresis and then transferred to a blotting membrane that is probed with target-specific antibody. Beginning with the transfer step, the entire process typically requires a minimum of 5 hours and up to 2 days to complete. VWR Life Science AMRESCO has developed novel reagents to streamline each step of the Western blotting process, enabling completion of the entire process in less than an hour and a half.

Central to the enhanced Western blotting system are Rapid Transfer Buffer, 10X and RapidBlock™ Solution, 10X. Available separately for use with any standard blotting protocol or as components of complete Rapid Western Blotting Kits, these reagents reduce transfer to 10 (semi-dry)–20 minutes (wet) and membrane blocking time to 5 minutes. The methanol-free formulation of Rapid Transfer Buffer offers transfer efficiency equivalent to that observed when using a standard tris-glycine-methanol buffer. It is compatible with conventional transfer equipment and both PVDF and nitrocellulose membranes. Non-specific binding of antibody to the blotting membrane is effectively blocked by protein-free RapidBlock Solution, resulting in blots with low background noise and clear results.

Rapid Transfer Buffer and RapidBlock Solution are bundled with additional reagents into kits for optimized Western blotting with target-specific antibodies from mouse, rabbit, goat, or rat hosts. Unique to Rapid Western blotting is the ability to combine primary and secondary antibody incubations into a single, 30 minute step. The primary antibody is mixed with Rapid Western Secondary-HRP diluted in Rapid Antibody Diluent, eliminating the need for more time-consuming, sequential incubation steps and intervening washes. A fast set of post-incubation washes is followed by detection with any standard chemiluminescent or colorimetric HRP substrate.

MATERIALS AND METHODS

Cell Culture

K562 cells were cultured in RPMI 1640 supplemented with 10% fetal calf serum, 100µg/mL streptomycin, and 100U/mL penicillin. The cells were grown in a 37°C humidified incubator with 5% CO_2 until ready for harvesting.

Protein Isolation

K562 cells were resuspended in growth media, counted and transferred to centrifuge tubes, then pelleted and washed once with PBS. Protein was isolated from 5 x 10^6 cells per tube using VWR Life Science AMRESCO's Cytoplasmic/Nuclear Protein Enrichment Kit. Aliquots of protein were stored frozen until ready for use.

Protein Electrophoresis

Cytoplasmic proteins were resolved by SDS-PAGE using Fluorescent SPRINT NEXT GEL® 12.5% or by standard Laemmli gel electrophoresis (10%).

Protein Transfer

Semi-dry transfer of protein to PVDF with Rapid Transfer Buffer, 10X was performed

according to the product instructions, with constant voltage set at 25V and transfer time set at 10 minutes. When using Tris-glycine-methanol for semi-dry transfer, constant amperage was set at 400mA for 1 hour. Voltage, time, and temperatures used for wet transfer with Rapid Transfer Buffer, 10X and Tris-glycine-methanol were 75V/25 minutes/room temperature and 100V/1 hour/4°C, respectively.

Gel Staining

Post-electrophoresis gel staining was performed using ZiP™ Reversible Protein Detection Kit or using standard Coomassie® gel staining, followed by destaining in 40% methanol/10% glacial acetic acid. All gel images were captured using a Syngene G:BOX HR gel documentation system.

Western Blotting

Rapid Western Blots were blocked 5 minutes in RapidBlock Solution and then incubated 30 minutes in combined primary and Rapid Western Secondary-HRP antibodies diluted in Rapid Antibody Diluent. The blots were then washed briefly with Rapid Wash Solution before detection with VisiGlo™ Plus HRP Chemiluminescent Substrate. Conventional Western blots were blocked in TBS-5% non-fat dry milk for 1 hour, followed by incubation in primary antibody diluted in blocking solution for 1 hour to overnight. The blots were washed and incubated with HRP-conjugated secondary antibody for 1 hour. After a final wash, the protein was visualized by VisiGlo™ Plus HRP Chemiluminescent Substrate. All blot images were captured using a Syngene G:BOX HR gel documentation system. Protein targets were probed using anti-ß-tubulin (1, 5,000, Epitomics), anti- ß-actin (1:1,000 Rockland Immunochemicals) and anti-NFκß p65 (1:1,000, Epitomics).

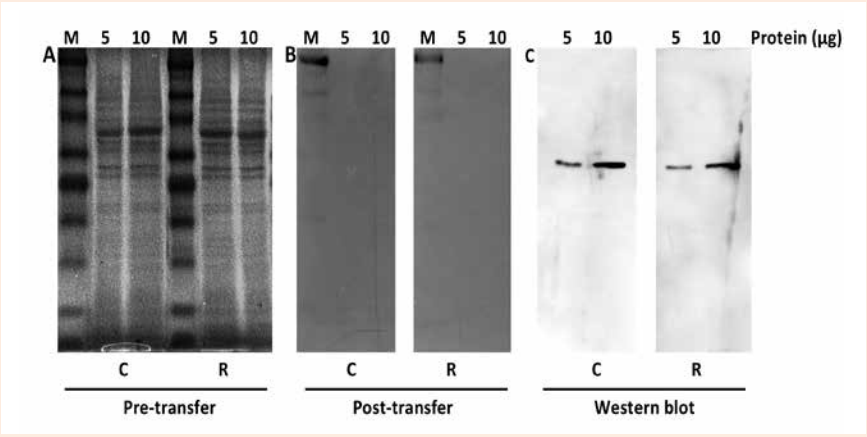


Figure 1. Comparison of Conventional and Rapid Transfer. Cytoplasmic protein samples (5µg and 10µg) isolated from K562 cells were resolved by 10% Laemmli gels at 150V for 1.5 hours. The gels were stained with ZiP Reversible Protein Detection Kit (A, Pre-transfer) and then wet transferred at 100V and 4°C for 1 hour with conventional, methanol-based transfer buffer (C) or at 75V and room temperature for 20 minutes with Rapid Transfer Buffer (R). The post-transfer gels were Coomassie stained and destained (B, Post-transfer). The PVDF membranes were probed with anti-ß-tubulin antibody (1:5,000, Eptiomics), followed by anti-Rabbit-HRP antibody (1:5,000, Rockland Immunochemical) using a conventional Western blotting procedure (C, Western blot). The blots were developed with VisiGlo™ Plus HRP Chemiluminescent Substrate and show very similar staining. Lanes marked with M represent BlueStep™ Broad Range Markers.

RESULTS AND DISCUSSION

Western blotting is routine for many labs, despite the considerable time it takes to perform. VWR Life Science AMRESCO sought to streamline the blotting process using innovative reagents that are compatible with standard equipment and handling processes. Rapid Transfer Buffer reduces the time required for the first step of blotting; transfer of protein from gel to membrane. Rapid and conventional Tris-glycine-methanol transfers were evaluated by first reversibly staining the post-electrophoresis gels to ensure they were equivalent before transfer by wet (Figure 1A) and semi-dry methods (data not shown). After destaining of the gels, one was wet transferred for 1 hour at 100V at 4°C in Tris-glycine-methanol, while the other was transferred for 20 minutes in Rapid Transfer Buffer at room temperature. The post-transfer gels were stained with Coomassie (Figure 1B) and the membranes were blotted with anti-ß-tubulin (1:5,000) antibody using a conventional Western blotting procedure (Figure 1C). The Coomassie stained gels and blotting results were very similar for both the conventional and Rapid transferred samples, indicating comparable transfer efficiencies were obtained. The same results were obtained using both transfer buffers with semi-dry blotting (data not shown), for which gels

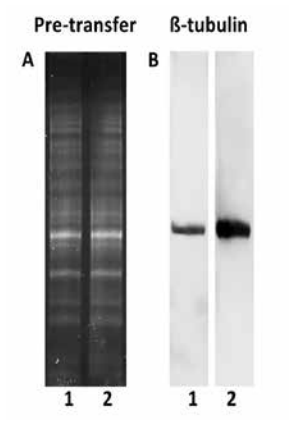


Figure 2. Blocking with RapidBlock™ Solution versus 5% non-fat milk. Cytoplasmic protein samples (10µg) isolated from K562 cells were resolved by 12.5% Fluorescent SPRINT NEXT GEL® and then visualized by UV transillumination (A) before transfer to PVDF membrane. The membrane was divided into strips that were blocked 1 hour in TBST/5% non-fat milk (B, lane 1) or 5 minutes in RapidBlock Solution (B, lane 2). The blots were probed with C-tubulin (1:5,000, Epitomics) antibody and HRP-conjugated secondary before detection with VisiGlo™ Plus HRP Chemiluminescent Substrate.

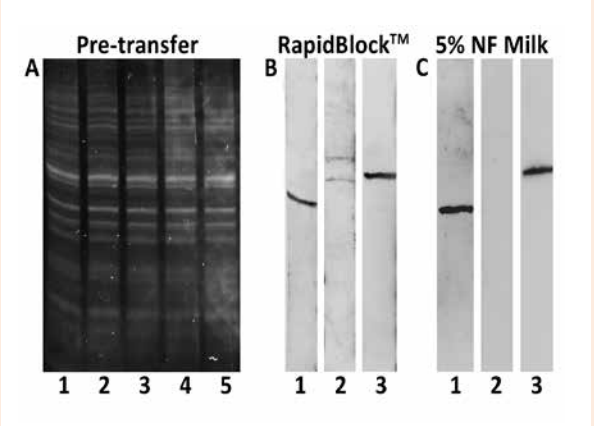
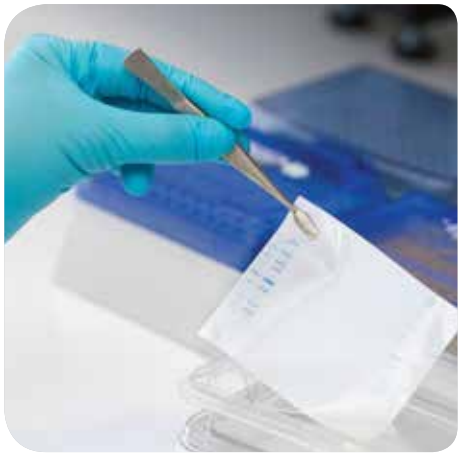


Figure 3. Rapid Western and conventional Western blotting comparison. Cytoplasmic protein samples (10µg) were resolved by 12.5% Fluorescent SPRINT NEXT GEL® and then visualized by UV transillumination (A) before transfer to PVDF membrane. The membrane was divided into strips that were probed with anti-ß-actin (1:1,000, Rockland Immunochemical, B1, C1), anti-NFκB p65 (1:1,000, Rockland Immunochemical, B2, C2) or anti- ß-tubulin (1:5,000, Epitomics, B3, C3) using a Rapid Western Blotting Kit (B) or conventional blotting (C).

were transferred 1 hour at 300mA in Tris-glycine-methanol and 15 minutes in Rapid Transfer Buffer at 25V.

Following protein transfer to PVDF or nitrocellulose, it is critical to block the membrane to prevent non-specific binding of antibody that causes high background noise during detection. Standard blocking is performed for 1 hour at room temperature or overnight at 4°C using non-fat milk or BSA. These common blocking reagents are proteins and thus have potential for cross-reactivity with the antibody probes. Despite its extensive use, non-fat milk is not recommended for use with biotinylated antibodies or detection of phosphoproteins because it contains



endogenous biotin and casein, a phosphoprotein. BSA is expensive and incompatible with lectin probes due to its carbohydrate content. To improve membrane blocking, RapidBlock™ Solution was developed as a protein-free solution that effectively blocks PVDF and nitrocellulose in 5 minutes. A comparison of blots blocked 5 minutes in RapidBlock (Figure 2B, Lane 2) and 1 hour in TBST/5% non-fat milk (Figure 2B, Lane 1) revealed clear signals for ß-tubulin (Figure 2) with low background . The signal was greater for the RapidBlock treated membrane, however, because of lower cross-reactivity with antibody. Protein-free RapidBlock Solution consistently performed as an effective and faster alternative to conventional membrane blocking with multiple antibodies tested in parallel with milk blocked blots (data not shown).

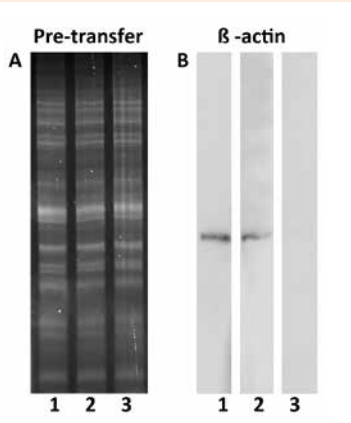


Figure 4. Rapid Western compared to a competitor's one-hour Western blotting kit. Cytoplasmic protein samples (10µg) were resolved by 12.5% Fluorescent SPRINT NEXT GEL® and then visualized by UV transillumination (A) before transfer to PVDF membrane. The membrane was divided into strips that were probed with anti-ß-actin (1:1,000, Rockland Immunochemical, B) by conventional Western blotting (B1), Rapid Western Blotting (B2) and a competitor's one-hour Western blotting kit (B3).

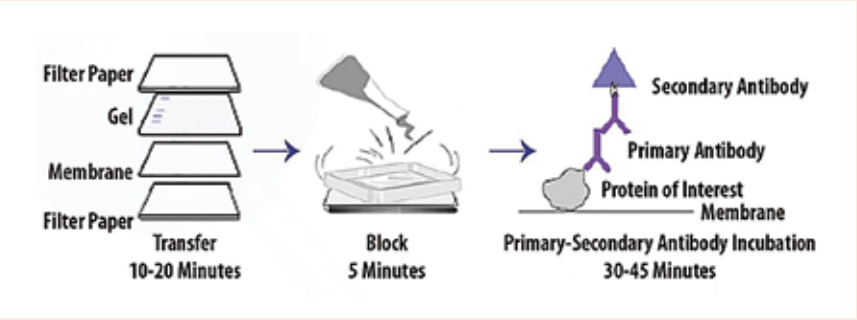


Figure 5. The Rapid Western blotting procedure.

Rapid Transfer Buffer and RapidBlock Solution are key components of Rapid Western Blotting Kits, which reduce the time for blotting to under an hour and a half, including transfer time. Time savings provided by the Rapid transfer and blocking reagents are enhanced further when used together with HRP-conjugated antibody and antibody diluent that allow for a combined primary-secondary antibody incubation step of just 30 minutes. To demonstrate the effectiveness of the Rapid Western, performance of the kit versus conventional blotting was tested using antibodies for ß-actin (Figure 3, B1, C1), NFκß p65 (Figure 3, B2, C2) and ß-tubulin (Figure 3, B3, C3). Both ß-actin and ß-tubulin were detected at similar levels with either blotting method, while NF-κß p65 was more apparent by Rapid Western blotting. Protein signals detected by Rapid Western blotting ranged between 100% and 60% when normalized to conventional blotting signals and were typically above 80%. Rapid Western blotting (Figure 4B, lane 2) was also evaluated for its performance versus a competitor's one-hour blotting kit (Figure 4B, lane 1), for which the one-hour begins with the blocking step. The signal on the Rapid Western blot (Figure 4B, lane 2) was comparable to the signal on a conventional blot (Figure 4, lane 1) and significantly more intense than the barely visible band obtained using the competitor's kit (Figure 4, B3). The evaluation repeated with other antibodies yielded similar results (data not shown), indicating this was not an experimental anomaly.

CONCLUSIONS

Although there are commercially available systems designed for fast transfer times and even "hands-off" blotting, these require costly investments in new equipment and consumable reagents. Rapid Western reagents were designed to maximize time savings without sacrificing the quality of the results using standard equipment available in the lab. Flexibility of Rapid Transfer Buffer and RapidBlock Solution used in combination with an otherwise conventional blotting procedure, in combination with each other, or as components of complete Rapid Western Blotting Kits makes it easy to find the right solution for your lab.

Description	Size	Cat. No.	Unit
Rapid Western Kit- Goat SDT	15 Blots	**71005-986**	**Each**
Rapid Western Kit- Goat WT	15 Blots	**71005-988**	**Each**
Rapid Western Kit-Mouse SDT	15 Blots	**97063-312**	**Each**
Rapid Western Kit- Mouse WT	15 Blots	**97063-316**	**Each**
Rapid Western Kit- Rabbit SDT	15 Blots	**97063-314**	**Each**
Rapid Western Kit- Rabbit WT	15 Blots	**97063-318**	**Each**
Rapid Western Kit- Rat SDT	15 Blots	**71005-990**	**Each**
Rapid Western Kit- Rat WT	15 Blots	**71005-992**	**Each**
Rapid Transfer Buffer, 10x	1 L	**97064-312**	**Each**
Rapid Transfer Buffer, 10x	4 L	**97064-314**	**Each**
Rapid Block Solution, 10x	15 mL	**97063-124**	**Each**
Rapid Block Solution, 10x	100 mL	**97064-124**	**Each**

Benefits of Using a Fluorescent Detection System in Quantitative Western Blotting Applications

Data quality in Western blotting or any process of protein analysis is essential for imaginative, forward-moving research. GE Healthcare Life Sciences is continuously innovating to help you achieve new standards in signal stability, detection sensitivity, protein quantitation, and optimization between reagents and instruments, including protein immunoblot detection systems and imagers.

Here we describe how various factors affect the final results in quantitative Western blotting applications and demonstrate the benefits of using a fluorescent detection system to reach precise quantitative data.

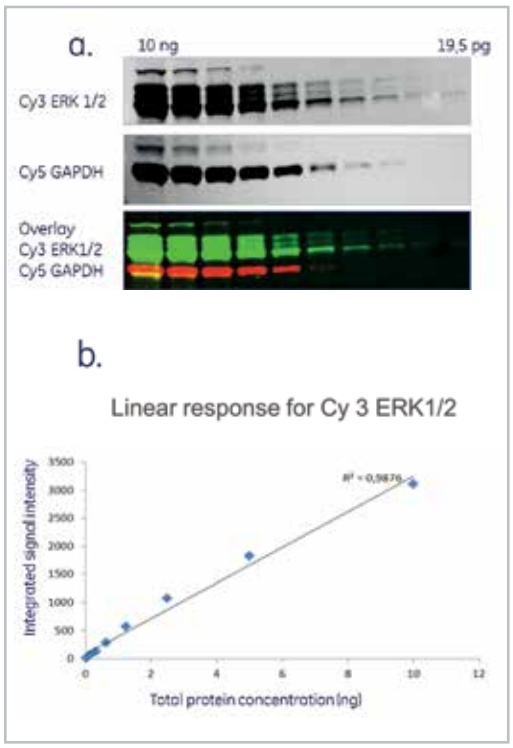


Figure 1. To identify low abundance proteins and quantify changes in expression levels it is necessary to utilize a detection system with high sensitivity and a broad linear dynamic range. Moreover, ability to discriminate among different signals enables multiplex detection, which simplifies and improves quantitative analysis. This experiment demonstrates high sensitivity (a) and broad linear dynamic range (b) in a multiplexed Western blotting for ERK 1/2 and GAPDH in a 2-fold dilution series of NIH/T3T cell lysate using Amersham™ ECL Plex™ and ImageQuant™ LAS 4010.

Western blotting is a well established and widely used technique to confirm the identity and presence of proteins from a variety of sources. In Western blotting, native or denatured proteins are first separated by gel electrophoresis according to molecular weight (under denaturing conditions) or by the three-dimensional structure of the protein (under native/non denaturing conditions). The separated proteins are then immobilized on a membrane, typically made of nitrocellulose or polyvinylidene difluoride (PVDF), where they are immunodetected by target specific antibodies.

For a long time, the technology was primarily used to confirm the presence or absence of a protein of interest in a complex sample. Lately, improved detection methods and imaging equipment have opened up the use of Western blotting as a tool for quantitative protein analysis. When performing quantitative Western blotting, it is important to consider sensitivity, signal stability, and the linear dynamic range provided by the detection system (the combination of detection reagent and imager). In addition, for accurate quantitation there is a prerequisite to perform in-lane normalization to correct for uneven sample loads. A description of how these various factors affect the quantitative results follows.

Sensitivity

Sensitivity, in the context of Western blotting, is defined as the minimum amount of protein that can be detected using available detection systems. Sensitivity can be affected by many factors, such as antibody affinity and concentration, detection method, and exposure

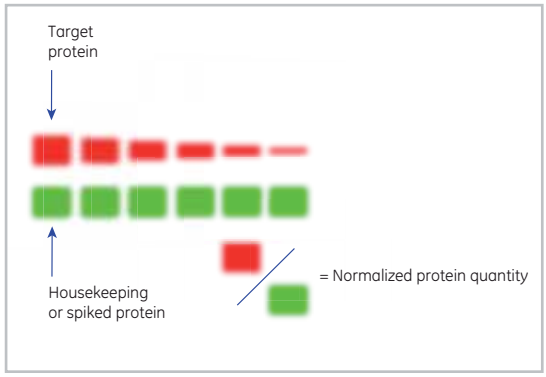


Figure 2. Illustration of how to normalize a target protein to a housekeeping or spiked protein. Initially, the intensity of each band is calculated using analysis software. The measured value for each target protein is then related to the corresponding value of the housekeeping or spiked protein.

Figure 3. The principle of Amersham ECL Plex. Primary antibodies against two proteins are recognized by species specific secondary antibodies conjugated to fluorescent Cy®Dyes, Cy3 and Cy5. Detection by direct fluorescence reduces the number of processing and imaging steps, which saves time and reduces errors in quantitation.

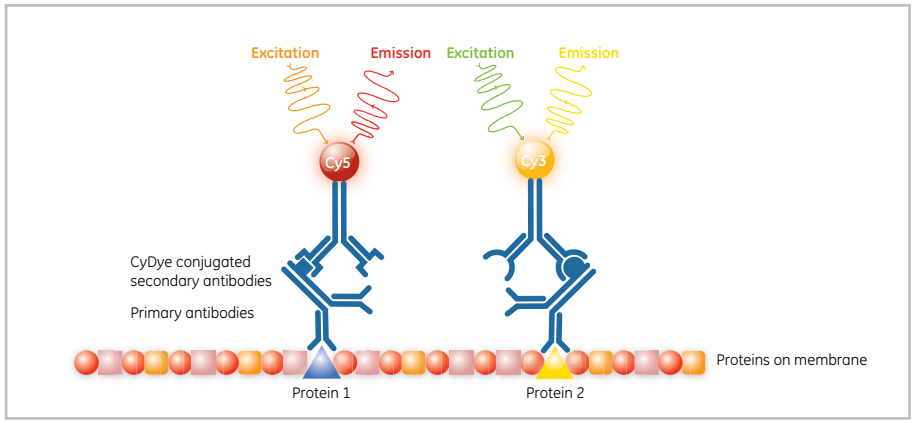
times. A highly sensitive detection system is necessary for accurate detection of minute changes in protein levels.

Linear Dynamic Range

The linear dynamic range occurs when the signal intensity is proportional to the protein quantity on a blot and thus allows precise quantitation throughout that range. This means that to obtain precise quantitation of strong as well as weak signals, the detection system must provide a broad linear dynamic range. If you have excessive amounts of protein, excessive concentrations of antibodies, or excessive exposure times, you may receive saturated signals. This means that the signal is no longer proportional to the amount of protein and the sample must therefore be excluded from the analysis.

Signal Stability

When performing quantitative Western blotting, it is advantageous to use a detection system with high signal stability, as this will impact the linear dynamic range and minimize signal variation. With a stable signal detection reagent, the time window for reaching high sensitivity is longer,



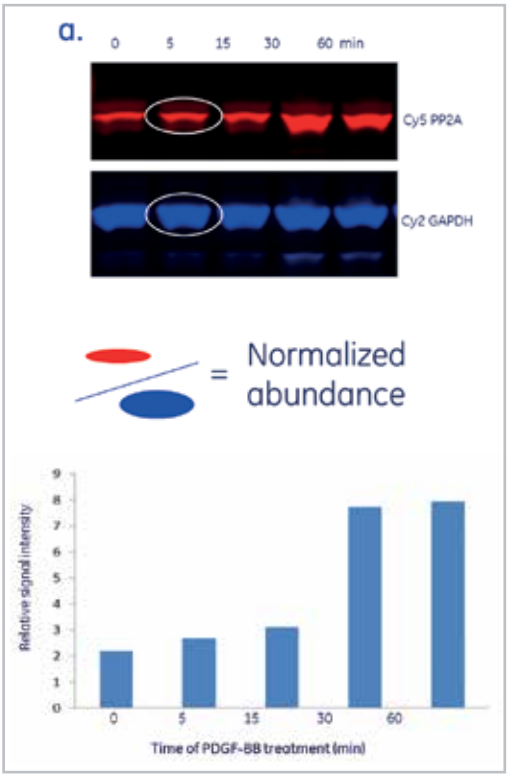


Figure 4. The target protein is the low expressed (PP2A) and housekeeping protein (GAPDH) are simultaneously detected in lysates from PDGF-BB treated cells, using Amersham ECL Plex Cy5 and Cy2. Levels of PP2A are normalized to GAPDH and quantified.

which then allows for multiple exposures and the possibility to detect weak bands that may be missed in a single, brief exposure.

In-lane Normalization

In order to reliably quantitate protein levels by Western blotting analysis, levels of the protein of interest should be normalized to an internal reference. This allows for variations in the amount of total protein from lane to lane due to errors such as inconsistent sample loading or, alternatively, different protein concentrations in the samples. If the sample is a cell lysate, an endogenous and unregulated "housekeeping" protein — a protein that is

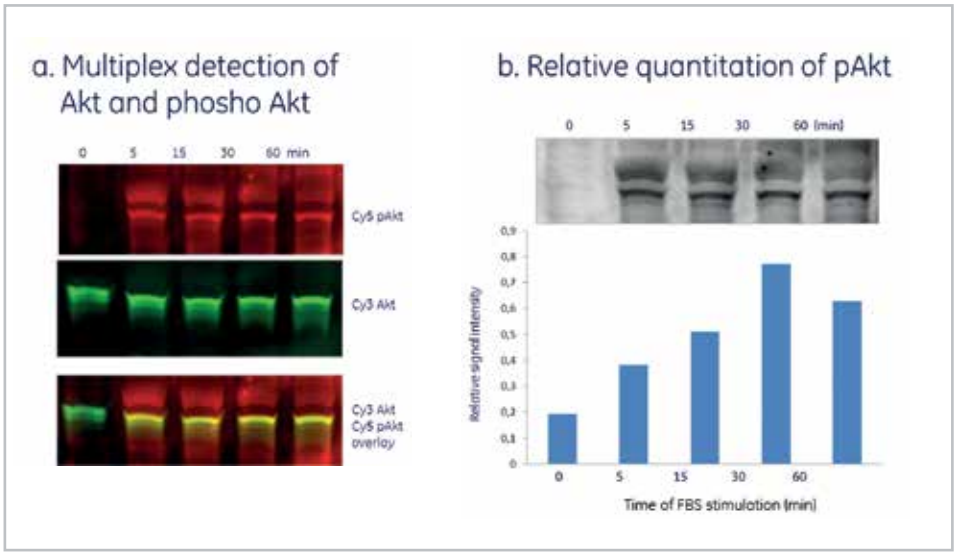


Figure 5. (a) Akt and phospho Akt in lysates from cells stimulated with 5% fetal bovine serum (FBS) at different time points. The proteins have overlapping sizes and the overlay image confirms phosphorylation since they appear at same size (resulting in yellow overlay). **(b)** Levels of phospho Akt are normalized to Akt and the quantification shows a time dependent phosphorylation of Akt as a response to the FBS stimulation.

expressed at a relatively constant rate and is also required for the maintenance of basic cellular functions — is commonly used as an internal standard.

Fluorescent Detection in Quantitative Western Blotting Applications

Fluorescent detection is a direct detection method where the secondary antibody is conjugated to a fluorophore. The obtained fluorescent signal is stable and directly proportional to the protein amount. Fluorescent detection systems are typically characterized by their high sensitivity and broad linear dynamic range, and are well adapted to quantitative Western blotting.

In the fluorescence-based Amersham ECL Plex system from GE Healthcare Life Sciences, discrete primary antibodies can be recognized by species specific secondary antibodies conjugated to fluorescent Amersham CyDyes. All CyDyes have their own specific excitation and emission wavelengths in the visible light spectra and are spectrally differentiated from each other, resulting in minimal cross talk. Thereby, up to three target proteins of overlapping sizes can be detected simultaneously. In addition, multiplex fluorescent Western blotting enables detection of both target proteins and housekeeping proteins on the same blot without stripping and re-probing, which simplifies normalization and improves quantitation.

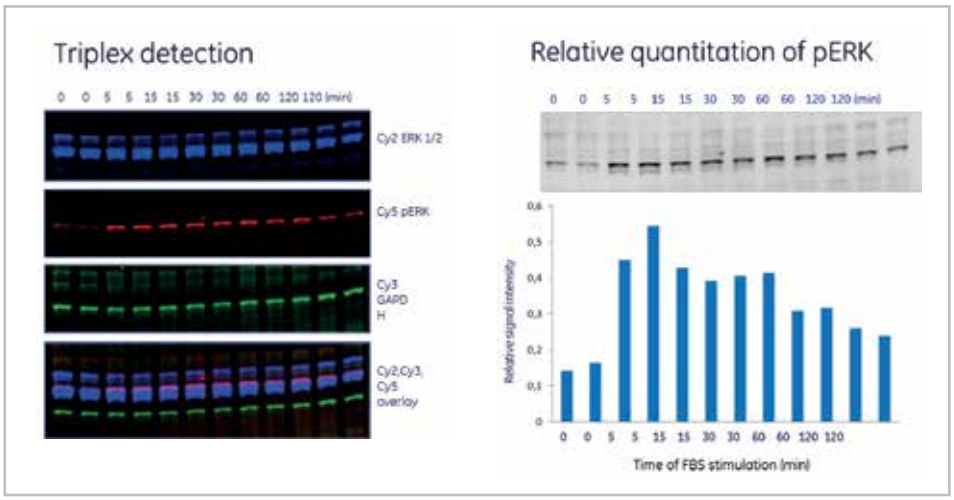


Figure 6. Utilizing Amersham ECL Plex Cy2, Cy3 and Cy5 it is possible to simultaneously detect three proteins on the same blot. This experiment demonstrates a triplex detection of ERK 1/2, phospho ERK (pERK) and GAPDH. ERK 1/2 and pERK are detected using Amersham ECL Plex Cy2 and Cy5 secondary antibodies while GAPDH is directly detected using a Cy3 labelled primary antibody. Levels of phospho ERK are normalized to ERK 1/2 and quantified to monitor phosphorylation levels as a response to serum stimulation. In-lane normalization of ERK 1/2 is done to GAPDH (not shown).

Distributor
GE Healthcare

The following section describes how fluorescence simplifies quantitation of protein levels when monitoring variation in protein expression as a response to various treatments in some typical Western blotting applications for cell signaling studies.

The first application demonstrates the advantage of using fluorescence for one step in-lane normalization by simultaneous detection of a target protein and a housekeeping protein. Here we were interested in monitoring the expression of PP2A in PDGF-BB stimulated cells over time. When looking at the expression levels of PP2A it appears that expression plateaus after 30 minutes and then slowly starts to decline. However, after in-lane normalization to GAPDH it is clear the decrease is an effect of uneven sample amounts in the various lanes.

Post translational modifications (PTMs) that do not change the molecular weight of a protein can be very difficult to study through traditional Western blotting detection techniques. They often require the blot to be stripped and re-probed, but this can lead to loss of protein and is therefore a risk when performing quantitative analysis. In this type of scenario, the potential of multiplexed detection provided by Amersham ECL Plex may be advantageous since both the non-modified and modified forms of the protein can be detected on the same membrane by using primary antibodies from two different species, which are then directed to the alternative forms of the protein. This is demonstrated in the second application where the spectral difference between Amersham ECL Plex Cy5 and Cy3 is utilized for multiplex detection of the non-phosphorylated and the phosphorylated forms of the protein. In this application the phosphorylation of tyrosine residues on Akt protein was analyzed and quantified after stimulation of serum starved cells with 5% fetal bovine serum. A time dependent increase in tyrosine phosphorylation of Akt was seen up to 30 minutes after stimulation with FBS. The results demonstrate how multiplexed detection with Amersham ECL Plex enables two epitopes on a single protein to be identified and simultaneously quantitated on a single Western blot.

The last example demonstrates how fluorescence can be used for detecting three different proteins simultaneously. This experimental set up has a great potential for monitoring the variation of protein expression for two proteins being part of the same signalling pathway, either with similar or different molecular weight. In this specific triplex application the expression level of non-phosphorylated and phosphorylated forms of ERK 1/2 are monitored together with a housekeeping protein over time in serum stimulated cells. The results demonstrate a fast increase in the phosphorylated form of ERK 1/2 when the serum starved cells are stimulated with serum.

Conclusions

Multiplexed detection by a fluorescence detection reagent like Amersham ECL Plex can improve the quantitative potential of Western blotting. It is useful for all types of quantitation, as stripping is not required to monitor levels of a housekeeping protein. This property makes it very useful to study PTMs that do not change the molecular weight of a protein. In addition, fluorescence detection is highly sensitive, covers a broad linear dynamic range, and emits a stable signal — all of which are factors that contribute to the quantitative strengths of the technique.

Description	Packaging	Cat. No.	Unit
Amersham Hybond LFP, PVDF, 0.2mm, 254 mm x 4 m	Roll	**10120-024**	**Pk. 1**
Amersham Hybond LFP, PVDF, 0.2µm, 200 x 200 mm	Sheets	**10120-078**	**Pk. 10**
Amersham Hybond LFP, PVDF, 0.2µm, 80 x 90 mm	Sheets	**10120-196**	**Pk. 25**
Amersham ECL Plex Goat-α-Mouse IgG-Cy3	150 µg	**95040-042**	**Each**
Amersham ECL Plex Goat-α-Rabbit IgG-Cy3	150 µg	**95040-030**	**Each**
Amersham ECL Plex Goat-α-Mouse IgG-Cy5	150 µg	**95040-046**	**Each**
Amersham ECL Plex Goat-α-Rabbit IgG-Cy5	150 µg	**95040-050**	**Each**
Amersham ECL Prime Blocking Agent	40 g	**95040-068**	**Each**
Amersham ECL Plex Fluorescent Rainbow Marker	120 µL	**95040-080**	**Each**
Amersham ECL Plex Fluorescent Rainbow Marker	500 µL	**95040-082**	**Each**

Western Blot Normalization:

Total Protein Normalization Using Stain-Free™ Gels and the Azure cSeries Imaging Systems

Total protein normalization (TPN) of Western blots is an alternative to the common practice of normalizing to housekeeping proteins. In TPN, the Western blot signal for the protein of interest in each lane is normalized to the total protein amount in the same lane. Stain-Free gels provide a quick way to assay total protein. The Azure cSeries instruments and AzureSpot software have built-in functionality for TPN of Western blots using Stain-Free gel technology.

INTRODUCTION

TPN allows Western blot normalization without needing to identify a housekeeping protein appropriate for a specific experiment or to optimize a second antibody to detect a housekeeping protein. To detect total protein for TPN many methods can be used. Stain-Free gel technology offers almost immediate detection of protein bands in gels and on transfer membranes without using colorimetric or fluorescent stains.

Stain-Free gels contain a trihalocompound within the gel matrix that produces a fluorescent product when covalently crosslinked via UV light to protein tryptophan residues. The resulting crosslinked products fluoresce under UV light and protein bands can be detected using an imaging system equipped with a UV illumination source such as the Azure cSeries Imaging Systems. With Stain-Free technology, gels can be imaged almost immediately after the completion of electrophoresis. Additionally, the crosslinked product can be detected on the membrane after protein transfer from the gel, allowing the transfer efficiency to be monitored by imaging the gel before transfer and the membrane after transfer.

The Stain-Free chemistry is compatible with downstream Western blotting. Here we describe carrying out TPN of a chemiluminescent Western blot using Stain-Free technology to assess total protein amount, using the Azure c600 and AzureSpot software.

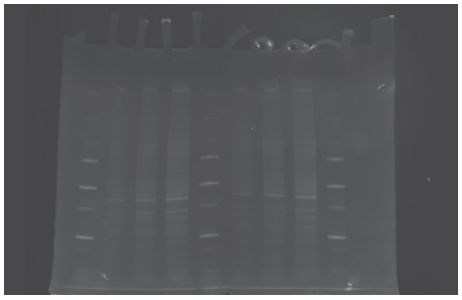


Figure 1. Total protein on SDS-PAGE gel. The AzureSpot c600 was programmed to expose the gel to UV light on the transilluminator for crosslinking followed by automatically capturing an image of the fluorescent product.

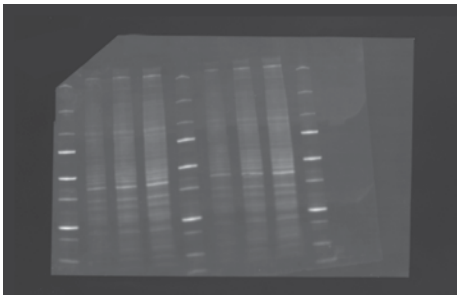


Figure 2. Image of total protein on the PVDF membrane after protein transfer. The membrane was imaged for 1 minute immediately after transfer.

METHODS AND RESULTS

Protein samples consisting of different amounts of the same HeLa cell lysate were separated by SDS-PAGE on a 4-15% Mini-PROTEAN® TGX Stain-Free Precast Gel. Immediately after completion of electrophoretic separation, the gel was placed directly on the UV transilluminator of an Azure c600 for activation and imaging. To activate the stain-free gel, the gel was exposed to UV light (302nm) for 5 minutes. An image of the crosslinked gel was then captured. The resulting gel image is shown in Figure 1.

The proteins from the gel were then transferred to a PVDF membrane. The membrane was activated by incubation with methanol for 15 seconds, water for 5 minutes, and then 1X Azure Transfer Buffer for 5 minutes. Transfer was performed using Azure Transfer Buffer at 19V for 35 minutes with an Idea Scientific GENIE electrophoretic transfer device.

Immediately after transfer was complete, the wet blot was placed directly on the UV transilluminator for imaging without rinsing. The blot was imaged on the UV transilluminator using UV light (302nm) for 1 min. The image is shown in Figure 2.

After imaging, the membrane was blocked for 30 minutes at room temperature. The membrane was then incubated for 1 hour with 2µg mouse anti-GAPDH antibody (EMD Millipore) in blocking buffer. The membrane was rinsed two times quickly with 1X PBST then washed three times, for 5 minutes each wash, with 1X PBST. The membrane was then incubated for 30 minutes with an HRP-conjugated goat anti-mouse antibody in blocking buffer and the membrane was washed as before.

Radiance ECL chemiluminescent substrate (Azure) was applied to the blot for 5 minutes. Excess substrate was drained from the membrane and the blot was placed in a black tray for imaging (Figure 3).

To normalize the amount of GAPDH detected by Western blotting to the total protein in each sample, the signal for the GAPDH band in each lane in the chemiluminescent image was normalized to the total protein amount detected in the stain-free image of the membrane for that lane.

First, a multiplex image was created from the stain-free image of the membrane and the chemiluminescent image of the GAPDH Western blot in AzureSpot Image Editor. AzureSpot software's guided workflow was used to identify lanes, conduct background correction, and identify GAPDH bands.

TPN is a normalization option in AzureSpot software. To carry out TPN, the

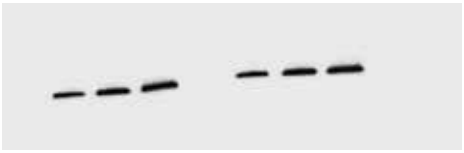


Figure 3. Chemiluminescent detection of GAPDH.

Normalization function was selected from the top menu (Figure 4). Total Protein Normalization was selected, and the channel with the stain-free image of the membrane was selected as the Normalization Channel. The Reference Lane was set to be lane 1. The software calculates a normalization factor for each lane based on the total protein signal in that lane compared to the reference lane. The normalized results for each lane are reported relative to the intensity of the band in the reference lane. Choosing a different reference lane will have no effect on the relative results, though the absolute numbers reported for the normalized bands will change.

The resulting unnormalized and normalized values for the GAPDH band were plotted (Figure 5). Though the amounts of sample loaded varied between lanes, the normalized values for GAPDH levels using TPN are identical across samples.

CONCLUSION

Azure Biosystems' imaging systems and software make total protein normalization of Western blots easy and efficient. TPN provides a simple way to normalize quantitative Western blots. Using

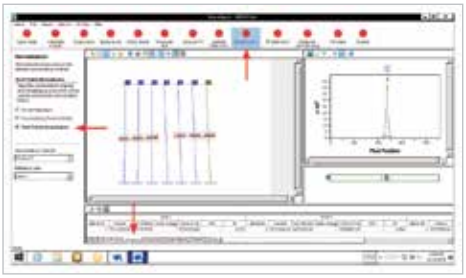


Figure 4. Total protein normalization in AzureSpot. TPN is a normalization option in the AzureSpot software. Simply select the normalization method and indicate which channel contains the total protein reference data.

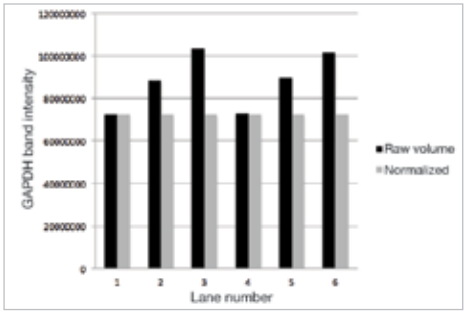
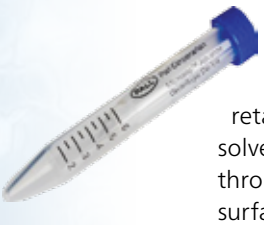


Figure 5. GAPDH band intensity before and after TPN. TPN accurately normalizes the values, accounting for the differences in loading amounts between lanes.

Stain-Free gel technology, total protein on the membrane can be measured by Azure's cSeries instruments immediately after protein transfer without any extra staining or destaining steps. The c300 through c600 imaging systems are compatible with this approach. AzureSpot software includes TPN as a built-in normalization method, streamlining the analysis workflow. Scientists need only capture two images, the total protein image and the Western image, and the software guides the rest.

Description	Application	Cat. No.	Unit
c150	For Gel Imaging (not upgradeable)	10767-150	Each
c200	For Gel imaging	10147-222	Each
c300	For Chemi and Gel Imaging	10147-220	Each
c400	For RGB, Chemi, and Gel Imaging	10147-218	Each
c500	For IR, Chemi, and Gel Imaging	10147-216	Each
c600	For IR, RGB, Chemi, and Gel imaging	10147-214	Each

Centrifugal Devices:
Simplifying Nucleic Acid and Protein Sample Prep



Pall's centrifugal devices simplify many common nucleic acid and protein sample preparation procedures. These devices provide efficient concentration and salt removal of samples from 50µL to 60mL in just minutes.

Ultrafiltration (UF) is a membrane separation technique based on molecular size, although other factors, such as molecule shape and charge, can also play a role. Molecules larger than the membrane pores in the UF membrane will be retained at the surface of the membrane while solvent and smaller solute molecules can freely pass through. This molecular exclusion at the UF membrane surface leads to concentration of the protein solute in the retained fraction (the retentate) and can be recovered from above the membrane.

There are three generic applications for ultrafiltration:

1. Concentration. Ultrafiltration is a very convenient method for the concentration of dilute protein or DNA/RNA samples. It is gentle and does not shear DNA up to 100Kb or cause loss of enzymatic activity in proteins. It is also very efficient, typically offering > 90% recovery.

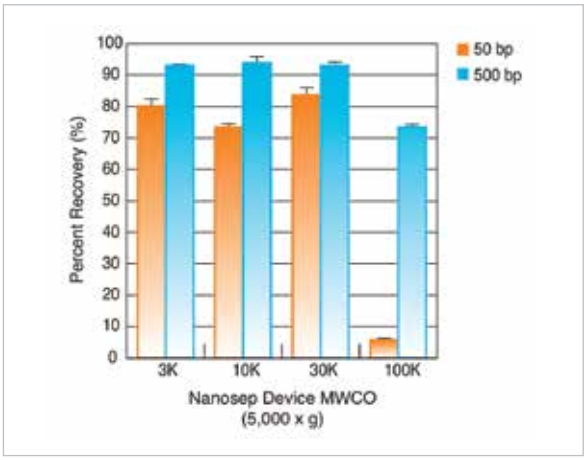
2. Desalting and Buffer Exchange (Diafiltration). Ultrafiltration provides a convenient and efficient way to remove or exchange salts, remove detergents, separate free molecules from bound molecules, remove low molecular weight components, or rapidly change the ionic or pH environment.

3. Fractionation. Fractionation using ultrafiltration is effective in applications, such as the preparation of protein-free filtrates, the separation of unbound or unincorporated label from DNA and protein samples, and the purification of PCR products from synthesis reactions.

Centrifugal devices can replace traditional separation techniques, such as column chromatography, preparative electrophoresis, alcohol or salt precipitation, dialysis, and gradient centrifugation when performing the following:

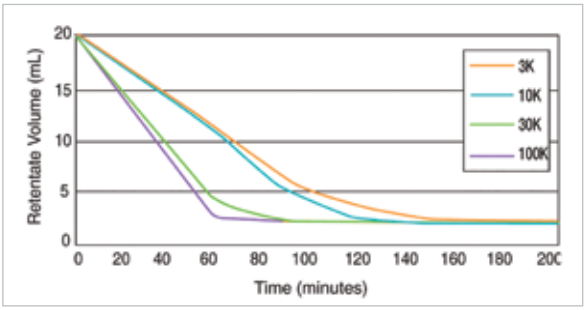
- Protein or nucleic acid concentration
- Desalting
- Buffer exchange
- Fractionation of protein mixtures
- Separation of primers from PCR products
- Separation of labeled nucleic acids or proteins from unincorporated nucleotides
- Virus concentration or removal
- Clarification of cell lysates and tissue homogenates

DNA Recovery as a Function of Device MWCO



A 500µL sample of a 100µg/mL DNA fragment solution containing 50 and 500bp double-stranded DNA fragments was centrifuged at 5,000 x *g* in Nanosep devices to a final volume of 50µL. Recovered samples were quantified using absorbance at 260nm. The 100K device was able to differentiate between the sizes of the DNA fragments.

Macrosep Advance Centrifugal Devices: Reduced Spin Time



Protein solutions were processed in each of the Macrosep Advance devices. Average time (minutes) is plotted against mL of remaining product to be filtered using a swinging bucket rotor at 5,000 x *g*. Solutions are 3K: Protamine Sulfate, 0.1% in 1X PBS, 10K: Cytochrome C, 0.025% in 1X PBS; 30K: IgG, 0.1% in 1x PBS; and 100K: Apoferritin, 0.1% in 1X PBS.

PALL Laboratory

Nanosep Centrifugal Devices

Simple, reliable concentrating and desalting of 50–500µL samples

- Ensures rapid processing of samples.
- Typical recoveries are > 90%. Available with low protein binding.
- Omega, Bio-Inert, and GHP membranes.
- A wide range of MWCOs, color-coded for easy identification.
- Constructed of low-binding polypropylene.

Macrosep Advance Centrifugal Devices

Quickly concentrates up to 20mL of biological sample without valuable sample loss

- Provides high recoveries, typically > 90%.
- Low protein-binding with Omega membrane.
- Versatile Omega membrane is available in a variety of MWCOs.
- Built-in deadstop prevents spinning to dryness.

Microsep Advance Centrifugal Devices

Precise, quick recovery of microliter volumes of concentrate

- High recovery. Achieve 50X concentration and > 90% recovery in just minutes.
- Features deadstop to prevent samples from spinning to dryness.
- Versatile Omega membrane is available in a variety of MWCOs.

Jumbosep Centrifugal Devices

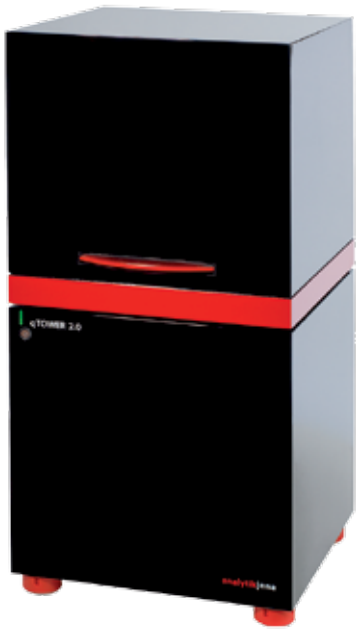
Convenient and reliable concentration, purification, and diafiltration of 15–60mL biological samples

- Provides high recoveries, typically > 90%.
- Low protein-binding with Omega membrane and polysulfone housing minimize losses due to non-specific binding.
- Versatile Omega membrane is available in a variety of MWCOs, color-coded for easy identification.
- Built-in deadstop prevents spinning to dryness.

MWCO	Cat. No.	Unit
NanoSep Centrifugal Devices		
3K	29300-606	Pk. 24
10K	29300-608	Pk. 24
30K	29300-610	Pk. 24
100K	29300-612	Pk. 24
MacroSep® Advance Centrifugal Devices		
1K	89233-882	Pk. 24
3K	89131-974	Pk. 24
10K	89131-980	Pk. 24
30K	89131-986	Pk. 24
100K	89131-992	Pk. 24

MWCO	Cat. No.	Unit
MicroSep® Advance Centrifugal Devices		
1K	89233-876	Pk. 24
3K	89132-004	Pk. 24
10K	89132-008	Pk. 24
30K	89132-012	Pk. 24
100K	89132-016	Pk. 24
JumboSep Centrifugal Devices Starter Kits		
3K	28148-044	Pk. 4
10K	29300-790	Pk. 4
30K	29300-792	Pk. 4
100K	28143-858	Pk. 4
300K	29300-796	Pk. 4

Thermal Shift Assay Using SYPRO® Orange and qTOWER 2.0/2.2 qPCR Thermal Cycler to Detect Protein Melting Temperatures



INTRODUCTION

The stability of proteins depends on ligand interactions, buffer conditions, or changes in conformation, which is traditionally investigated by time-consuming circular dichroism (CD) spectroscopy. The thermal shift assay is an alternative method for protein stability determination based on temperature-induced denaturation. It can be monitored, via qPCR by using an environmentally sensitive fluorescent dye such as SYPRO® Orange. When added to a protein solution, SYPRO Orange remains quenched as long as the proteins remain folded in an aqueous solution. As the temperature is increased, the proteins unfold exposing their hydrophobic core. The SYPRO Orange binds to the core releasing the fluorescence signal. The dye quenching effect initiated by water is reduced by thermal unfolding and the increasing fluorescence signal can be measured. The temperature in the middle of the thermal denaturation process is defined as melting temperature (Tm). Shifts of the Tm provide an indication of a change in protein stability.

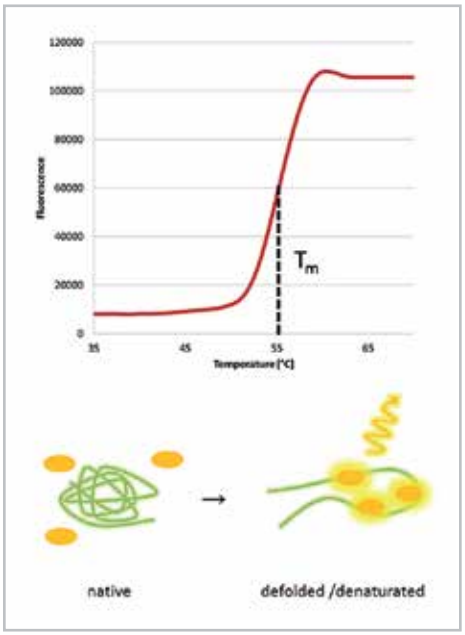


Figure 1. Melting curve of a protein detected by using qTOWER 2.2 in combination with SYPRO Orange.

The thermal shift assay is an alternative method for protein stability determination based on temperature-induced denaturation. It can be monitored, via qPCR by using an environmentally sensitive fluorescent dye such as SYPRO® Orange. When added to a protein solution, SYPRO Orange remains quenched as long as the proteins remain folded in an aqueous solution. As the temperature is increased, the proteins unfold exposing their hydrophobic core. The SYPRO Orange binds to the core releasing the fluorescence signal. The performance of a melting curve by using the qTOWER 2.0/2.2 real-time PCR thermal cycler thus allows an easy and fast determination of protein melting temperatures. The midpoint or melt peak of the generated melting curve corresponds to the melting temperature (Tm value) of the protein under current conditions.

Protein thermal shift assays are sensitive and rapid tools to examine protein thermal stability, helping to evaluate protein-ligand binding to find optimal buffer conditions or to analyze protein variations. In this experiment, the Tm values of α-Chymotrypsinogen A in TBS with three different NaCl concentrations were determined.

MATERIAL AND METHODS

Chemicals

- NaCl stock solution (5M and 0.2M in TBS buffer)
- SYPRO Orange (1:200)
- TBS buffer (10mM)

Instruments

- qTOWER 2.0/2.2 with Color Module Protein 1 – SYPRO Orange (490nm/580nm)

Profile	Temperature	Holding	Ramp Rate
Equilibration	25°C	10sec	max.
Melting Curve*	25 - 90° and 6 sec. with ΔT = 1°C		

*Data acquisition: Color Module Protein 1 (490 – 580nm and Gain 5)

Table 1. Temperature and time protocol.

Component	20 mM NaCl	500 mM NaCl	2M NaCl
α-Chymotrypsinogen A	1 µl	59 µl	1 µl
TMS buffer	59 µl	59 µl	38 µl
NaCl solution (5 M)	_	7 µl	28 µl
NaCl solution (0.2 M)	7 µl	_	_
SYPRO® Orange	3 µl	3 µl	3 µl
Final Volume	70 µl	70 µl	70 µl

Table 2. NaCl dilutions in TBS contain 1mg/mL α-Chymotrypsinogen A.

NaCL	Tm	Mean Tm
20 mM	49.1°C	49.1°C
20 mM	49.2°C	
20 mM	49.0°C	
500 mM	51.4°C	51.3°C
500 mM	51.1°C	
500 mM	51.4°C	
2 M	51.4°C	54.6°C
2 M	51.6°C	
2 M	51.7°C	

Table 3. Melting points of α-Chymotrypsinogen A.

UVP
An Analytik Jena Company

Each sample was measured in triplicate with 20µL per reaction. Additionally, a negative control was used for reference.

RESULTS AND DISCUSSION

Figures 3 and 4 show the melting curve of α-Chymotrypsinogen A and accordant analysis. The calculation and display of first derivatives of the melting curves is completed automatically by the qTOWER 2.0/2.2's qPCRsoft control and analysis software. The shift in Tm shows a clear influence of the different NaCl concentrations to the thermal stability of the protein.

When NaCl concentrations rise, the thermal stability of α-Chymotrypsinogen A rises as well. Higher salt concentrations lead to the formation of hydration shells surrounding the proteins, thus stabilizing α-Chymotrypsinogen A. Therefore, the melting point Tm is shifted by nearly 6°C from approx. 49°C at 20mM NaCl to approx. 55°C at 2M NaCl.

SUMMARY

In contrast to a traditional CD Spectroscopy assay which takes approximately 1 hour per sample, the current experiment was performed in 10 minutes for 9 samples with excellent resolution of the investigated melting point. Even in cases of using the maximum number of 96 samples, the experimental time remains the same, a huge advantage of using the qTOWER 2.0/2.2's technology for thermal shift assays. Furthermore, high reproducibility is due to simple procedures, the high precision of the qTOWER 2.0/2.2 qPCR system, and the striking sensitivity of SYPRO Orange.

Description	Cat. No.	Unit
qTOWER 2.0, 115V	10066-140	Each
qTOWER 2.2, 115V, with gradient function	10066-144	Each
Color module Protein 1 (for SYPRO Orange)	10066-166	Each

Additional color and FRET modules available.

Figure 2. Plate layout for melting curve analysis.

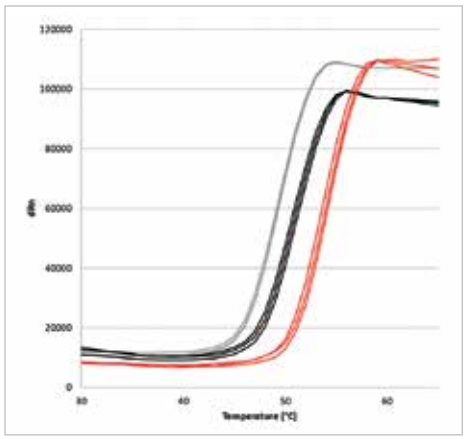


Figure 3. Melting curves of α-Chymotrypsinogen A under influence of different NaCl concentration: 20mM (grey), 500mM (black) and 2M (red).

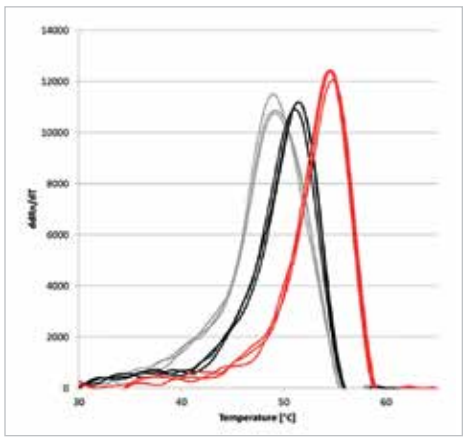


Figure 4. Melting curve derivatives of the thermal shift assay using SYPRO Orange and qTOWER 2.2; NaCl concentrations: 20mM (grey), 500mM (black) and 2M (red).

in vivo-jetPEI:

A Powerful, Polymer-Based Vector to Safely and Easily Deliver Nucleic Acids *in vivo*

Valérie Kédinger, Anne-Laure Bellemin, Patrick Erbacher

INTRODUCTION

The *in vivo* delivery of nucleic acids into animal models is essential for basic research, as well as for medical applications such as gene therapy. Viral vectors are efficient carriers, but their use is limited because of safety issues such as induction of immune response and virus-associated pathogenicity. These concerns have led to increased interest in non-viral methods for *in vivo* gene delivery.

The second generation of non-viral vectors offers improved performance and safety, making it a viable alternative to viral gene delivery. *in vivo*-jetPEI, a cationic, polymer-based reagent, is a very powerful, non-viral vector that safely and easily delivers nucleic acids through various *in vivo* administration routes. It offers high performance in terms of efficiency, reproducibility, and robustness. Currently, *in vivo*-jetPEI is the most widely used reagent to deliver genes in animals and has been selected as the delivery vector of choice in a growing number of preclinical studies and clinical trials for drug development programs worldwide.

a.

	30 min	24 h
4°C	45.6 ± 5.6 nm	47.1 nm ± 5.8 nm
RT	41.1 ± 12.0 nm	45.8 ± 12.9 nm
37°C	46.9 ± 8.9 nm	45.9 ± 13.8 nm

b.

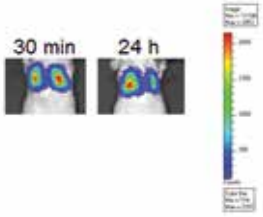


Figure 1. Complex stability at different storage temperatures. (a) Forty micrograms of pCMVLuc were complexed with *in vivo*-jetPEI (N/P=8). Complex size was determined after 30 min and 24h storage at RT, 4°C, and 37°C by Dynamic Light Scattering using a zeta sizer. (b) For *in vivo* imaging, complexes incubated 30 min or stored 24h at 4°C were intravenously injected through the retro-orbital sinus. The animals were imaged with the IVIS100® imaging system 24h after injection (exposition time 5s).

in vivo-jetPEI/Nucleic Acid Complex Characteristics

in vivo-jetPEI plays an important role in protecting nucleic acids from degradation by nucleases. Due to its high cationic charge density potential, this polymer-based reagent can condense any type of nucleic acid (DNA, siRNA, miRNA, oligonucleotides, etc.) to form stable complexes and promote gene expression or silencing in a wide range of tissues. The overall charge of the *in vivo*-jetPEI/nucleic acid complex is crucial, as only positively charged molecules can bind with the cell surface via interaction with negatively charged syndecans and then be transported into intracellular vesicles. Moreover, a small number of complexes is also essential to promote efficient gene delivery *in vivo*. The team at Polyplus-transfection optimized the conditions necessary to obtain the best delivery by testing different nucleic acid-to-reagent ratios (data not shown) and analyzed the stability of the complexes formed. To measure the stability of these complexes, *in vivo*-jetPEI/luciferase expressing plasmid complexes were prepared and measured with Dynamic Light Scattering 30 min after complexation or when stored 24h at different temperatures (room temperature (RT), 4°C, and 37°C). Figure 1a shows that *in vivo*-jetPEI/DNA complexes are stable for at least 24h at RT, 4°C, and 37°C, as no variation in size was observed. Luciferase expression in the lungs was determined by bioimaging the samples after storage of the complexes at 4°C for 24h and compared with data gathered after the 30 min complexation time. No significant variation of luciferase expression level was observed after 24h storage (Figure 1b). These data indicate that the complexes can be prepared in advance for a whole group of animals and then stored for many hours before use as they are stable in size, do not form aggregates, and allow an efficient delivery of nucleic acids.

Expression Profile Depending on the Route of Administration

The delivery of nucleic acid *in vivo* into animals presents a higher degree of complexity

Polyplus transfection™

compared to *in vitro* transfection since before entering the cells, the complexes first need to reach the target organ or tissue. The stability of the complexes formed with *in vivo*-jetPEI allows numerous routes of administration, which can then increase the number of available target organs. Figure 2 illustrates both intravenous and intraperitoneal injection delivery routes followed by analysis of luciferase gene expression in live animals (Fig 2a) or in extracted organs (Fig 2b). While intravenous injection allows a wide delivery to many organs with a preferred expression to lung, heart, and salivary glands, intraperitoneal injection is the administration route of choice to target the ovaries and pancreas. These data highlight the importance of adapting the delivery route according to the goal of the *in vivo* experiments and to the target organ.

Time Expression After *in vivo*-jetPEI/ Nucleic Acid Complex Injection

in vivo-jetPEI-mediated delivery of nucleic acids allows transient expression or silencing *in vivo*. Polyplus-transfection investigated the time course of the luciferase expression in lungs after intravenous injection using a Caliper bioimaging system. As shown in Figure 3a, bioimaging of whole animals shows lung luciferase expression as early as 12h after intravenous injection of nucleic acid/*in vivo*-jetPEI complexes. In lungs, maximum expression is observed between 12 and 24h after systemic injection (respectively 6.9×10^5 and 9.1×10^5 photons) with a sustained expression up to 72h after injection (Figure 3a). Lungs were dissected after imaging and the lung extracts were assayed for luciferase expression, confirming the results obtained in live animals (Figure 3b). The luciferase protein is not a very stable protein and its half life is quite short, explaining the relatively short time course of expression observed in this experiment. Using a different reporter protein, such as eGFP or delivering a plasmid without CpG motives in order to avoid the silencing of gene expression over time can lead to increased transgene expression duration.

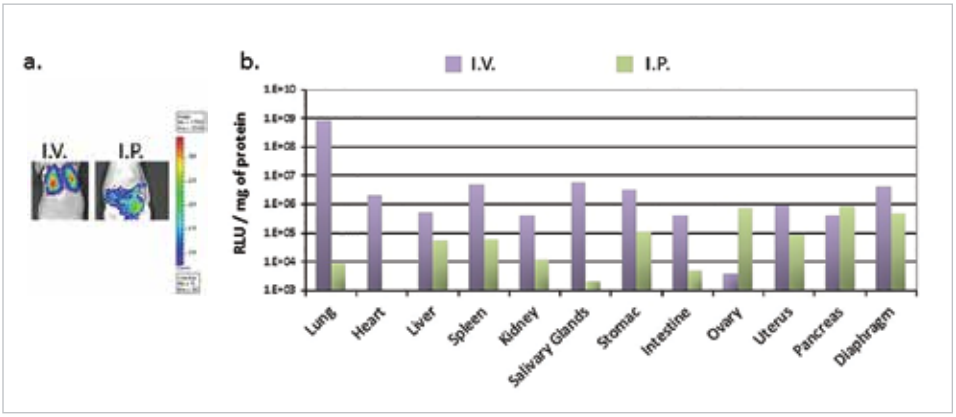


Figure 2. Expression profile depending on the administration route. (a) Forty micrograms of pCMVLuc complexed with *in vivo*-jetPEI (N/P=8) were either intravenously injected or intraperitoneally injected. Mice were imaged 24h after complex injection using the IVIS100® imaging system (exposition time 10s). (b) After imaging, different organs were removed and luciferase expression was assayed in organ extracts, n=6.

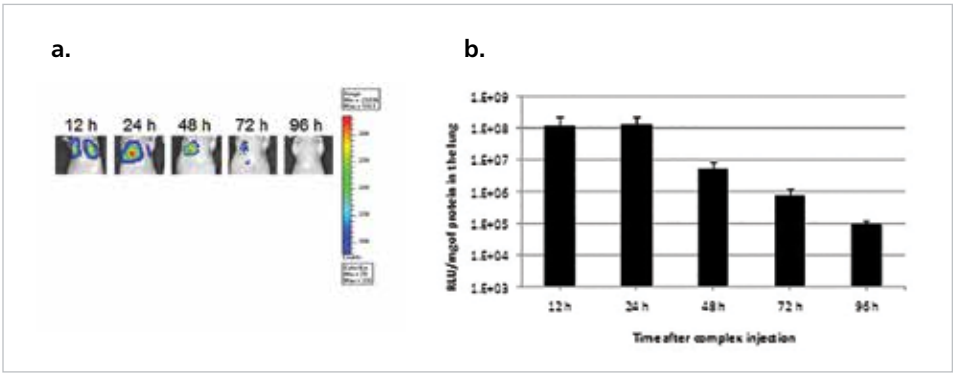


Figure 3. Luciferase expression time course after *in vivo*-jetPEI®/DNA complex injection. (a) Forty micrograms of pCMVLuc complexed with *in vivo*-jetPEI (N/P=8) were injected through the retro-orbital sinus. Mice were imaged 12, 24, 48, 72, and 96h after complex injection using the IVIS100® imaging system (exposition time 10s). (b) After injection, lungs were removed and luciferase expression was assayed in lung extracts, n=6.

CONCLUSION

in vivo-jetPEI-mediated gene delivery is a very useful and easy-to-develop technology. This delivery technology can be adapted to target different organs using various routes of administration including systemic injection or local delivery routes like intra-tumoral, intra-thecal, or subcutaneous. Taken together, the data shows that *in vivo*-jetPEI is a potent reagent for functional and therapeutic studies in animal models.

Size	Cat. No.	Unit
0.1 mL	89129-960	Each
0.5 mL	89129-962	Each

qScript™ microRNA Quantification System

Seamless, Sensitive microRNA Quantification with Simply Total RNA

Jonathan Doose, M.Sc.

microRNA (miR) is small subset of non-coding RNA that hybridizes with complementary sequences in mRNA to regulate gene-expression. This specific type of small non-coding RNA has been described in numerous human diseases and is rapidly gaining the attention of biomedical research scientists and medical oncologists as a potential diagnostic tool. Real-time quantitative PCR (qPCR) is the most sensitive technique for quantifying specific genetic sequences; however, miR are too small (19–22nt) for random hexamer priming and do not possess a poly(A) tail for priming by oligo(dT). Quantabio has developed a complete reagent system that enables sensitive and reliable quantification of miR with economical SYBR® Green I dye instead of costly hydrolysis (Taqman®) probes.

Quantabio microRNA System Overview

The qScript microRNA cDNA Synthesis kit (Figure 1) contains optimized reagent components for efficient polyadenylation and reverse transcription of small ssRNA across a broad spectrum of RNA input (10pg–1µg). qScript reverse transcriptase technology drives high-yield first-strand cDNA using optimized 2-step, single-tube reaction chemistry. The resultant cDNA possesses a qPCR-optimized adapter sequence in the oligo(dT) primer that enables seamless downstream quantification by qPCR. Compared with stem-loop RT priming methods that reverse transcribe miRs individually in separate reactions and provide only 6–8bp of miR sequence complementarity, the Quantabio approach enables hundreds of miRs to be profiled and compared directly within the same cDNA sample.

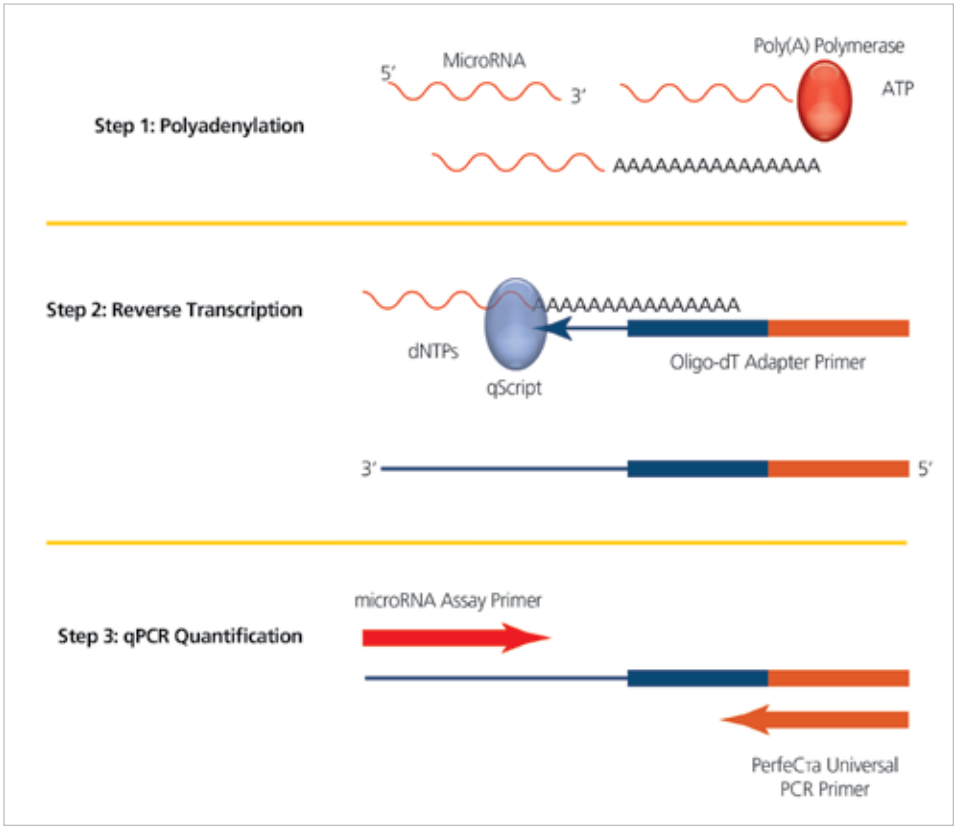


Figure 1. Quantabio microRNA Quantification System: Small single-stranded RNAs are first polyadenylated by poly(A) polymerase and then reverse transcribed by qScript in a single, 2-step reaction. The resultant cDNA possesses a qPCR-optimized adapter sequence that enables seamless qPCR quantification with PerfeCTa® SYBR Green SuperMixes and user-defined forward primer.

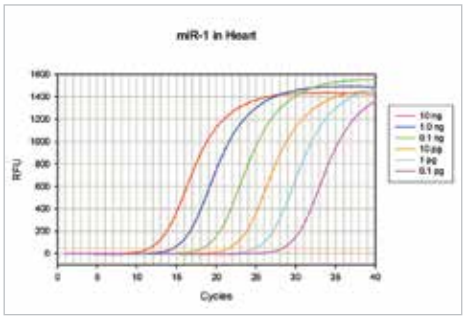


Figure 2. Detection of Rare microRNAs (miR-1): The Quantabio microRNA profiling system provides linear detection and quantification of miRs across total RNA input levels spanning six orders of magnitude.

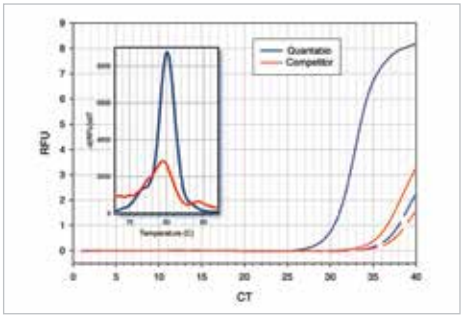


Figure 3. Robust Yields and Confident Controls (miR-1247): qScript drives high yields of first strand cDNA synthesis in a single, 2-step reaction that enables minus poly(a) polymerase controls (dashed lines) to clearly distinguish between mature miR and precursor pri-miR.

Quantabio
We put the Q in qPCR

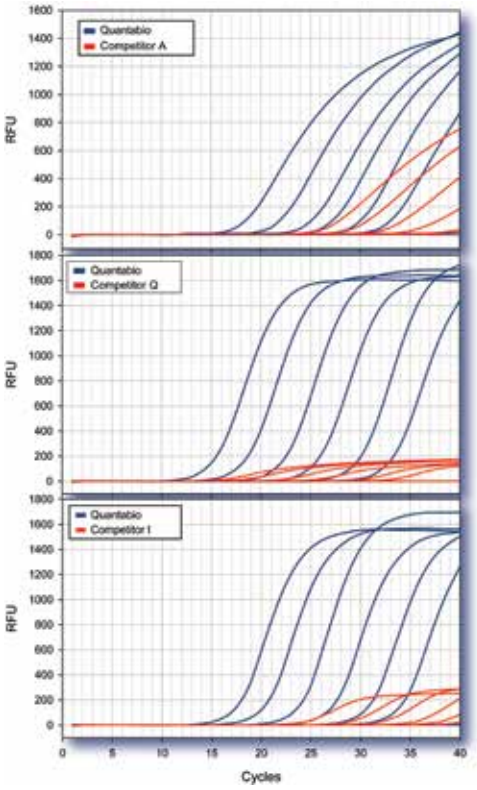


Figure 4. Reliable Assay Performance
The Quantabio microRNA profiling system yields superior results when compared to other leading miR quantification systems. PerfeCTa miR Assays are specific to sequences in miRbase and wet-lab validated by Quantabio scientists. Top panel: miR-124, middle panel: let-7a, bottom panel: miR-27a.

Sensitive microRNA Detection across a Broad Range of Total RNA Input

Engineered, ultrapure enzymes are the basis of every Quantabio kit. The Quantabio microRNA Quantification system will detect low copy miRs more reliably than other systems and provides confident assay results with scarce biological specimens or rare miRs (Figure 2, 4).

Confident Controls Strengthen Results

Unlike other supplier kits, the Quantabio reagent system provides additional resolution between mature miRs and precursor pri-miRs with a simple minus poly(A) polymerase (PAP) control (Figure 3). This is not possible with reagent systems that pre-blend PAP and RT enzymes together. Each kit also includes a positive control for SNORD44 that may also be used as a calibration target to normalize qPCR input amounts between RNA samples.

Reliably Robust qPCR Assays

Quantabio reagent technology delivers consistently robust assay performance and reliable sensitivity for rare miRs when compared to other kit suppliers (Figure 4). PerfeCTa® SYBR Green SuperMixes contain ultrapure, high-yielding hot start DNA polymerase, formulated with a maximal concentration of intercalating dye to ensure robust signals from small PCR amplicons (e.g., miR templated cDNA). PerfeCTa reagents are also formulated with proprietary anti-foam additives that eliminate persistent bubbles and reduce intra-assay variability allowing precious samples to be maximized.

Description	Rxns, µL	Cat. No.	Unit
qScript microRNA cDNA Synthesis Kit	25 x 20	**89168-788**	**Each**
qScript microRNA cDNA Synthesis Kit	100 x 20	**89168-790**	**Each**
PerfeCTa SYBR Green SuperMix	250 x 20	**101414-150**	**Each**
PerfeCTa SYBR Green SuperMix	1250 x 20	**101414-152**	**Each**
PerfeCTa SYBR Green SuperMix	5000 x 20	**101414-146**	**Each**
PerfeCTa SYBR Green SuperMix, LR	250 x 20	**101414-166**	**Each**
PerfeCTa SYBR Green SuperMix, LR	1250 x 20	**101414-168**	**Each**
PerfeCTa SYBR Green SuperMix, LR	5000 x 20	**101414-162**	**Each**
PerfeCTa SYBR Green SuperMix, R	250 x 20	**101414-158**	**Each**
PerfeCTa SYBR Green SuperMix, R	1250 x 20	**101414-160**	**Each**
PerfeCTa SYBR Green SuperMix, R	5000 x 20	**101414-154**	**Each**
PerfeCTa Universal PCR Primer	500 x 20	**89168-792**	**Each**

E.Z.N.A.® Plant DNA DS Kit

OMEGA bio-tek
Innovations in nucleic acid isolation

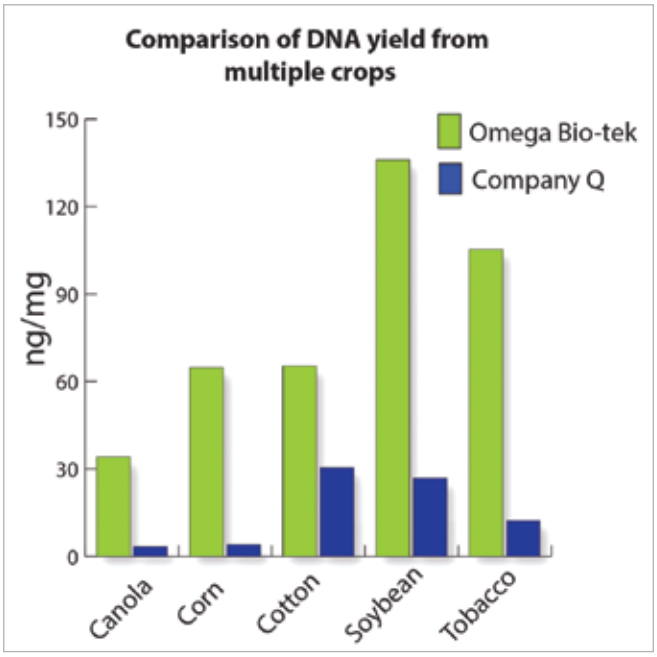
Isolate Genomic DNA from Leaf and Seed Tissue Containing High Amounts of Polysaccharides and Polyphenols

The E.Z.N.A. Plant DNA DS Mini Kit is designed for maximum recovery of genomic DNA up to 30kb in size from fresh, frozen, or dried plant tissue samples rich in polysaccharides or polyphenols, or those samples with a lower DNA content. Up to 50mg wet tissue can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of the HiBind® matrix with the speed and versatility of spin column technology to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from plant tissue lysates.

This procedure relies on the well established properties of the cationic detergent, cetyltrimethyl ammonium bromide (CTAB), in conjunction with the unique binding system for increased yields of high-quality DNA. The system eliminates the need for chloroform extractions traditionally associated with CTAB based lysis methods. Samples are homogenized and lysed in a high salt buffer containing CTAB. Binding conditions are adjusted and DNA is purified using a HiBind® DNA Mini Columns. Salts, proteins, and other contaminants are removed to yield high-quality genomic DNA suitable for downstream applications such as endonuclease digestion, thermal cycle amplification, and hybridization applications.

Features

- **Speed —** Includes homogenizer columns
- **Quality —** Pure DNA for downstream applications such as qPCR and next generation sequencing
- **Versatile —** Isolate DNA from a wide variety of plant sample types



40–50mg of various fresh leaf tissue were extracted in triplicate according to the manufacturer's protocol and eluted to 100µL. DNA was analyzed with a fluourescent DNA-based quantification method. Total yield was divided by total tissue amount to show ng of DNA per mg of leaf tissue.

Description	Preps	Cat. No.	Unit
E.Z.N.A Plant DNA DS Kit	5	**10017-798**	**Each**
E.Z.N.A Plant DNA DS Kit	50	**10017-800**	**Each**

Improved Library Quantitation for a Broad Range of Library Types Using the NEBNext® Library Quant Kit for Illumina®

Nathan A. Tanner, Ph.D., Janine G. Borgaro, Ph.D., Erbay Yigit, Ph.D., Don Johnson, Ph.D., Julie F. Menin, Eileen T. Dimalanta, Ph.D. and Nicole Nichols, Ph.D.

Accurate quantitation of a next generation sequencing (NGS) library is essential for maximizing data output and quality from each sequencing run. qPCR is widely accepted as the most effective method for library quantitation, as it measures only sequenceable library fragments with a high level of accuracy and consistency. The NEBNext Library Quant Kit for Illumina from New England BioLabs® offers a simple, robust, qPCR-based method for the quantitation of libraries to be sequenced on the Illumina platform.

Here we demonstrate the effectiveness of the NEBNext Library Quant Kit for a broad range of library types and sizes, while also highlighting the advantages offered by qPCR quantitation for obtaining optimal cluster density and performance consistency.

RESULTS

For a method or kit to be a trusted way to quantitate libraries, the values obtained must not only be accurate, but also consistent, both between libraries and between users.

qPCR Accuracy

Electrophoretic quantitation methods such as the Agilent Bioanalyzer® instrument can provide information on library size, but for quantitation this method can be less accurate and consistent. Also, electrophoretic methods quantitate all DNA molecules present in a library, in contrast to qPCR which quantitates only molecules with an adaptor ligated to each end.

In this experiment, quantitation values were obtained for multiple replicates of libraries of different size and GC content (Figure 1). Concentrations of four libraries were determined by the NEBNext Library Quant Kit (orange) and compared to values measured by the Bioanalyzer (blue). Compared to NEBNext qPCR, Bioanalyzer concentrations displayed a greater level of variation, demonstrating the benefits of qPCR for library quantitation.In addition to the method itself, the consistency of the reagents involved and ease-of-use of the protocol both minimized variability. In a separate experiment, replicates of 340–400bp libraries from *E. coli, H. influenzae,* and human (IMR-90) genomic DNA were quantitated by four different users with the NEBNext or Kapa™ Library Quant Kit (Universal). While both kits were able to successfully quantitate the various sample types, a marked improvement in quantitation consistency was observed using the NEBNext Library Quant Kit (data not shown).

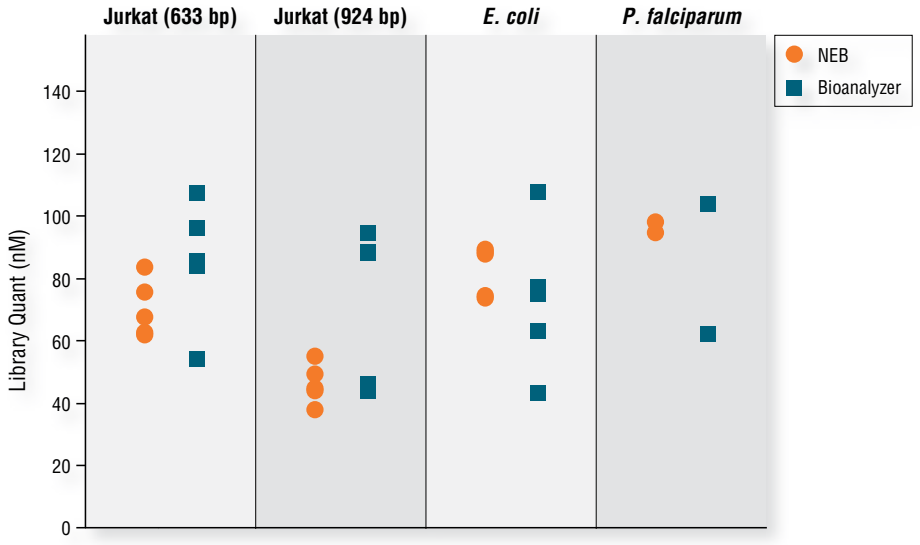


Figure 1. qPCR provides more consistent library quantitation results than Bioanalyzer analysis: Concentrations of four libraries were determined by the NEBNext Library Quant Kit (orange) and compared to values measured using the Agilent Bioanalyzer (blue).

Correlation with Cluster Density

The most relevant measure of accuracy in library quantitation is the density of clusters achieved after loading the recommended amount of library. If the quantitation value is too low, more

library will be loaded and over-clustering will result. In contrast, if the quantitation value is too high, less library than desired will be loaded, producing under-clustered samples. When seven different libraries at a range of concentrations were quantitated using the NEBNext Library Quant Kit, then diluted to 8pM and loaded into cluster generation, a raw cluster density average of 1160k/mm^2 was measured (data not shown). This falls directly in the optimal range of 900–1300k/mm^2.

Quantitation of a Broad Range of Libraries

The consistent and reliable performance of a method or kit across a variety of libraries, in terms of GC content and insert size, is critical for practical utility. The ability of the NEBNext Library Quant Kit to accurately quantitate a wide range of library types was tested by using libraries from ten different sources, including human and microbial DNA, with high GC and high AT content, and including a broad range of library sizes (150 – 963bp) (Figure 2). In all cases, optimal cluster density was achieved using concentrations determined by the NEBNext Library Quant Kit.

CONCLUSION

The NEBNext Library Quant Kit provides accurate and reliable qPCR-based library quantitation of Illumina libraries, as shown by the production of optimal cluster densities. The NEBNext kit demonstrates improved reproducibility and consistency when compared to alternative methods and kits. Furthermore, this kit can successfully quantitate libraries from a wide variety of sample types, as well as a broad range of sizes and GC-content.

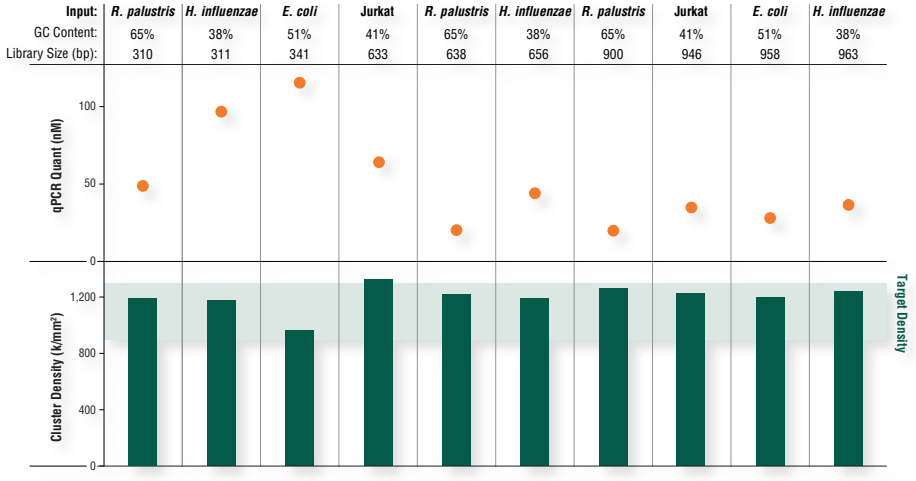


Figure 2. NEBNext Library Quant Kit delivers accurate quantitation for a variety of sample types and sizes: Libraries of 310–963bp from the indicated sources were quantitated using the NEBNext Library Quant Kit, then diluted to 8pM and loaded onto a MiSeq (v2 chemistry; MCS v2.4.1.3). Library concentrations ranged from 7–120nM, and resulting raw cluster density for all libraries was 965–1300k/mm^2 (ave. =1199). Optimal cluster density was achieved using concentrations determined by the NEBNext Library Quant Kit for all library sizes.

US ONLY

Description	Rxns	Cat. No.	Unit
NEBNext Library Quant Kit for Illumina	100	**102942-190**	**Each**
NEBNext Library Quant Kit for Illumina	500	**102942-192**	**Each**
NEBNext Ultra II DNA Library Prep Kit for Illumina	24	**102969-110**	**Each**
NEBNext Ultra II DNA Library Prep Kit for Illumina	96	**102969-108**	**Each**
NEBNext Ultra Directional RNA Library Prep Kit for Illumina	24	**102715-920**	**Each**
NEBNext Ultra Directional RNA Library Prep Kit for Illumina	96	**102715-918**	**Each**
NEBNext Multiplex Oligos for Illumina (Index Primers Set 1)	24	**102500-100**	**Each**
NEBNext Multiplex Oligos for Illumina (Index Primers Set 1)	96	**102500-098**	**Each**
NEBNext Multiplex Oligos for Illumina (Index Primers Set 2)	24	**102715-934**	**Each**
NEBNext Multiplex Oligos for Illumina (Index Primers Set 2)	96	**102715-932**	**Each**
NEBNext Multiplex Oligos for Illumina (Methylated Adaptor, Index Primers Set 1)	24	**102877-574**	**Each**
NEBNext Multiplex Oligos for Illumina (Methylated Adaptor, Index Primers Set 1)	96	**102877-572**	**Each**
NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)	96	**102877-580**	**Each**

Products not available in Canada

Performance Improvements for High Resolution Anion-Exchange Oligonucleotide Separations Using Small Particle Substrates

Thermo SCIENTIFIC

J.R. Thayer, G. Gendeh, S. Rao, D. Jamieson, C.A. Pohl, and Y. Agroskin

INTRODUCTION

Anion-exchange chromatography (AEC) on pellicular resins has delivered industry-leading oligonucleotide (ON) resolution for over 20 years. Introduction of the DNAPac PA100 in the early 1990s provided for facile separations of closely related sequences, even of the same lengths. The second generation of the DNAPac PA200 (2004) increased throughput and column longevity, especially at high pH values.[1]

Described here is the newest generation column, the DNAPac PA200 RS, which further improves resolution at the very highest levels,[2-4] or improves throughput when good resolution has already been achieved. This new column is best used with chromatographs capable of very high pressures and that harbor low-system volumes.

METHODS

Sample Preparation

ON samples were acquired from Integrated DNA Technologies (Coralville, IA). Dry ONs were suspended to 1.5 or 6mg/mL in DI water and diluted in DI water as needed.

Liquid Chromatography

Separations were performed on Thermo Scientific™ Dionex™ UltiMate 3000 BioRS Systems, each consisting of a:

- LPG-3400RS Quaternary Rapid Separation Pump
- WPS-3000RS Rapid Separation Wellplate Sampler
- TCC-3000RS Rapid Separation Thermostatted Column Compartment
- VWD-3400RS Rapid Separation Four Channel Variable Wavelength Detector with PEEK semimicro flow cell **or**
- DAD-3000RS Rapid Separation Diode Array Detector with PEEK semimicro flow cell. For isocratic column performance analyses, a Thermo Scientific™ Dionex™ ED50A Electrochemical Detector with a 2mm or 4mm Thermo Scientific™ Dionex™ ASRS-300 Anion Self-Regenerating Suppressor was used with a DS3 Detection Stabilizer (conductivity cell).

Data Analysis

Chromatographic system control, data acquisition, and peak integration were managed via the Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, Version 6.8 (Table 1).

RESULTS

I. Column Performance

Performance goals were to deliver: 1) a column that produces resolution comparable to the standard DNAPac PA200, but with significantly higher throughput; and 2) a column with significantly better resolution in a timeframe comparable to that used for standard DNAPac columns. The first tests compared isocratic and gradient column performance between a standard (4 × 250mm) DNAPac PA200 and a shorter (4.6 × 150mm) DNAPac PA200 RS column (Figure 1).

The DNAPac PA200 RS column, although shorter, delivered better efficiency than the 8µm DNAPac column and in comparable time. On a plate per meter basis, the smaller particle RS column delivered more than twice the efficiency of the standard column, and with identical selectivity.

Under these gradient conditions, the DNAPac PA200 RS produced an average of 21% increased resolution over the 8µm DNAPac column, even in the shorter format, and in 35% less time (Figure 2).

II. Applications

Most ON separations attempt to resolve related sequences on the basis of length, and the DNAPac performs this function well. Annealing of single-stranded RNAi sequences into duplexes is known to generate 2′,5′-linkages,[2,3] and phosphorothioate linkages generate diastereoisomers.[4] Resolution of these forms is often critical for therapeutic ON developments (Figure 3).

In this comparison, the DNAPac PA200 RS column provides comparable resolution for all three pairs of ONs in less than 75% of the time required for the standard DNAPac PA200. Due to the smaller particle size, the

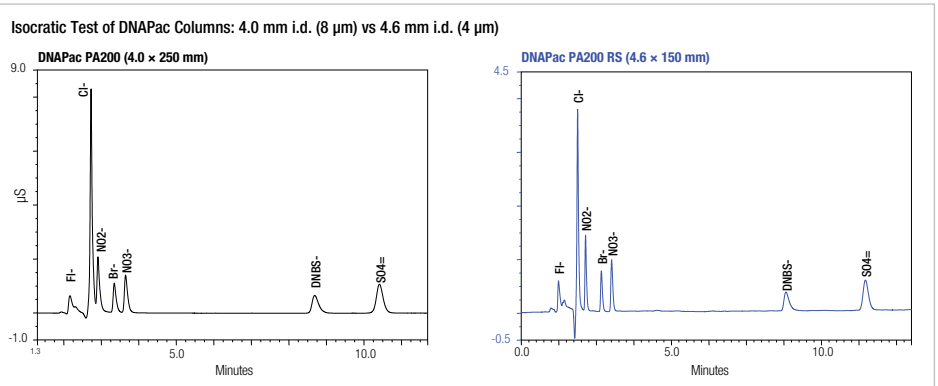
RNA

Dio-1: 5′-AUG AAC UUC AGG GUC AGC UUG -3′ Dio-6: 5′-AUG AAC UUC A*G*G GUC AGC UUG -3′
Dio-9: 5′-AUG AAC UUC AGG GUC* AGC UUG -3′ eGFP-S: 5′-AGC UGA$_s$ CCC UGA AGU UCA UdCdT-3′

DNA

dT19-24: 5′-TTT TTT TTT TTT TTT TTT T(T,T, T,T,T) -3′
ON34: 5′-TAG GTT CTC TAA CGC TGA CTG ATT GTA GGT GTT C- 3′
ON35: 5′-GTA GGT TCT CTA ACG CTG ACT GAT TGT AGG TTC TC- 3′
ON44: 5′-TGA CTG ATT GTA GGT TCT CTA ACG CTG ACT GAT TGT AGG TTC TC - 3′
ON45: 5′-CTG ACT GAT TGT AGG TTC TCT AAC GCT GAC TGA TTG TAG GTT CTC - 3′
ON54: 5′-TCT GTA ACG CTG ACT GAT TGT AGG TTC TCT AAC GCT GAC TGA TTG TAG GTT CTC - 3′
ON55: 5′-TTC TGT AAC GCT GAC TGA TTG TAG GTT CTC TAA CGC TGA CTG ATT GTA GGT TCT C - 3′

Table 1. ONs used (* indicates 2',5'-linkage; $_s$ indicates PS linkage).

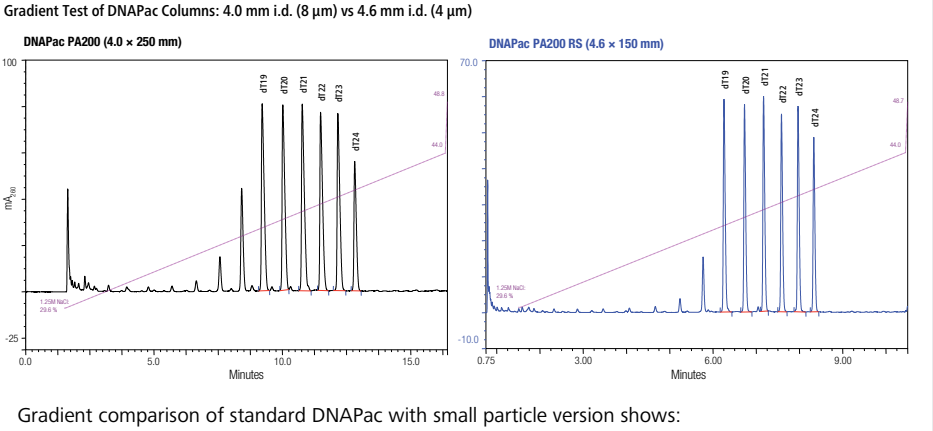


Isocratic comparison of DNAPac with small particle version shows:

- Similar retention
- Good asymmetry
- Improved efficiency
- Identical selectivity

Column #	Diam. Mm	Length mm	Temp °C	Flow	Inj Vol (µL)	tr (SO4=)	N	Asym	dP (psi)	tr DNBS /SO4=	Plates/m
DNAPac PA200	4.0	250	30	850	31	10.74	12228	1.53	1288	0.77	48912
DNAPac PA200 RS	4.6	150	30	1120	25	11.47	19349	1.23	5265	0.77	128993

Figure 1. Isocratic elution of seven small anions employed 1.7mM $NaHCO_3$ and 1.8mm Na_2CO_3 at a linear velocity of 1.12mm/s using a Dionex ASRS-300 to suppress background signal.



Gradient comparison of standard DNAPac with small particle version shows:

- Reduced retention
- Improved peak width
- Improved resolution
- Appropriate selectivity

Column #	Flow (µL/min)	d-PSI (Max)	Asym (dT23)	tr (dT23)	PW-dT23 (5%, CV)	Rs (19/20)	Rs (21/22)	Rs (22/23)	tr ratio 22/23
DP200 SN 3265	850	1655	1.51	10.92	0.056	4.8	4.4	4.1	0.94
DNAPac PA200 RS	1060	47.6	1.85	7.97	0.048	6.1	5.3	4.8	0.95

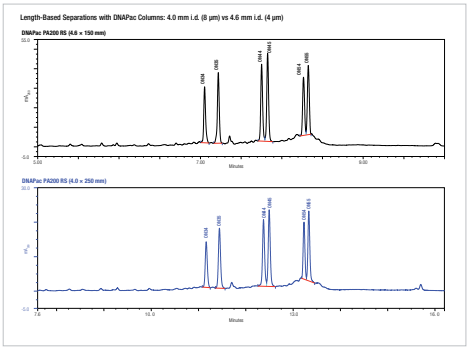


Figure 3. ONs composed of 34, 35, 44, 45, 54, and 55 bases were injected and eluted with a gradient of 370–550mm NaCl over 4 column volumes in Tris-buffered eluent at pH 8 and 30 °C. Linear velocity: 1.12mm/sec. Top Panel: DNAPac PA200 RS, 4.6 × 150 mm. Bottom Panel: standard DNAPac PA200, 4.0 × 250mm.

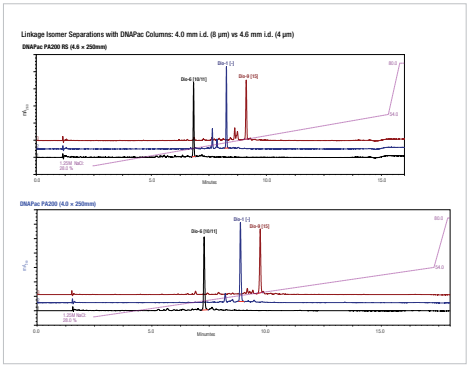


Figure 4. Three 21-base identical sequence ONs with or without 2′,5′-linkages in specific positions within the sequence were chromatographed with a gradient of 350–675mm NaCl over four column volumes in Tris-buffered eluent at pH 8 and 30°C. Top Panel: DNAPac PA200 RS, 4.6 × 250 mm. Bottom Panel: Standard DNAPac PA200, 4.0 × 250mm. Pressure for RS column: 8800psi (600 bar).

Figure 2. (left) Gradient elution of six deoxythymidine ONs (19–24 bases) using a gradient of 370–550mm NaCl in Tris buffer at pH 8 and 30 °C. The linear velocity was 1.12mm/s and absorbance detection was at 260nm.

RS column also delivers better detection sensitivity (Figure 4).

Here, the 4.6 × 250mm DNAPac PA200 RS demonstrates better resolution at higher throughput even with identical column lengths. The throughput for the RS column is ~10% higher and peak widths are better than for the DNAPac PA200 (Figure 5).

Each of these 4.6mm i.d. columns resolved the two diastereoisomers arising from the phosphorothioate linkage at position 6 in the eGFP-S sequence. Even the 50mm long format generated a resolution >4 for these isomers, and does so in <3 min, showing the power of the pellicular anion-exchange approach.

II. Column Ruggedness

There are few high-performance columns designed for ONs, and most exhibit degradation within a relatively low number of sample injections. The DNAPac PA200 RS operates at elevated pressures (up to 11,000 psi, 750 bar) and exhibits very good column longevity (Figure 6). Little, if any, column degradation was observed over the course of this test. In addition, tabulation of all of these samples (Table 2) reveals RSD values for retention time and asymmetry of <0.15% and 1.0%, respectively.

CONCLUSIONS

It was confirmed that the DNAPac PA200 RS column family meets the following criteria:

- In 150mm format, greater throughput, efficiency, and resolution comparable to

Thermo SCIENTIFIC

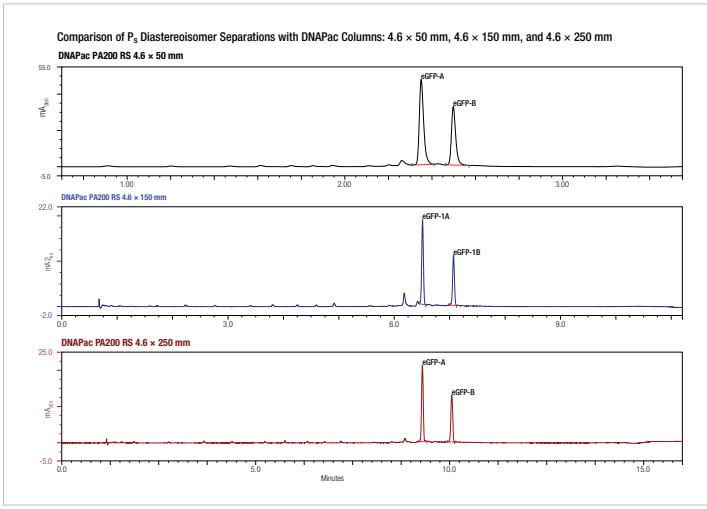


Figure 5. A 21-base ON (eGFP-S) was chromatographed on the DNAPac PA200 RS in three lengths: 50, 150 and 250mm. In each case the diastereoisomers are readily resolved from one another. The 250mm column completed the separation in ~10.5 min, the 150mm column resolved the isomers within 7.5 min, and the 50mm column completed the separation in 2.6 min. These separations are difficult with other chromatographic modes. Conditions are the same as in Figure 4 for RS column.

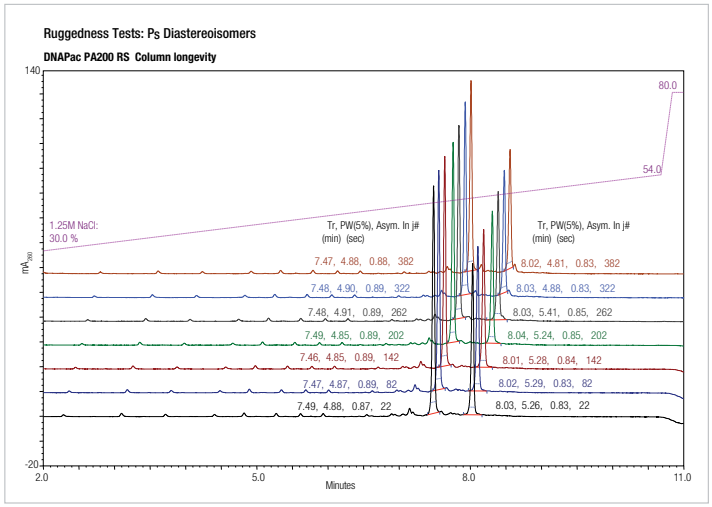


Figure 6. Here, a 150mm-long DNAPac PA200 RS column was run for >350 gradient cycles with injection of different sets of ONs each 20 cycles. The gradient was 350–675mm NaCl in six column volumes at pH 8 and 30°C at a linear velocity of 1.12mm/sec. At this flow the maximum pressure was 5800psi (400 bar).

the standard 4 × 250mm DNAPac PA200 (Figures 1, 2, and 3).

- In 250mm format, better throughput and resolution than the standard 4 × 250mm DNAPac PA200 (Figure 4).
- In common applications, such as resolution of "n" from "n-1" length ONs, results are demonstrated in less than 8 min, even for fairly long sequences (Figure 3).
- In difficult ON separations, such as resolution of linkage and diastereoisomers, results are achieved (Figures 4 and 5), even with formats only 50 mm in lengths (Figure 5).
- The small-particle column exhibits very good ruggedness, maintaining very small peak widths for over 350 injections.

References

1. Thayer, J.R., Barreto, V.; Rao, S.; Pohl, C.A. Control of Oligonucleotide Retention on a pH- Stabilized Strong Anion Exchange Column. Anal. Biochem. *2005, 338. 39–47.*

2. Thayer, J.R., Rao, S.; Puri, N.; Burnett, C.A.; Young, M. Identification of Aberrant 2´-5´ RNA Linkage Isomers by Pellicular Anion Exchange Chromatography. Anal. Biochem. *2007, 361, 132–139.*

3. Thayer. J.R., Puri, N.; Burnett, C.; Hail, M.; Rao, S. Identification of RNA Linkage Isomers by Anion Exchange Purification with Electrospray Ionization Mass Spectrometry of Automatically Desalted Phosphodiesterase-II Digests. Anal. Biochem. *2010, 399, 110–117.*

4. Thayer, J.R., Wu, Y.; Hansen, E.; Angelino, M.D.; Rao, S. Separation of Oligonucleotide Phosphorothioate Diastereoisomers by Pellicular Anion-Exchange Chromatography. J. Chromatogr. *A 2011, 1218, 802–808.*

Inj #	Sample Name	eGFP-S(A) Ret.Time min	PW (5%) sec	Asym(EP)	eGFP-S(B) Ret.Time min	PW (5%) sec
2	eGFP-S	7.501	5.03	0.87	8.05	5.41
22	eGFP-S	7.485	4.88	0.87	8.03	5.26
42	eGFP-S	7.458	4.93	0.88	8.01	5.34
62	eGFP-S	7.473	4.91	0.89	8.03	5.43
82	eGFP-S	7.468	4.87	0.89	8.02	5.29
102	eGFP-S	7.467	4.91	0.89	8.02	5.31
122	eGFP-S	7.467	4.82	0.88	8.02	5.3
162	eGFP-S	7.498	4.84	0.89	8.05	5.23
182	eGFP-S	7.487	4.86	0.89	8.04	5.26
202	eGFP-S	7.49	4.85	0.89	8.04	5.24
222	eGFP-S	7.486	4.84	0.89	8.03	5.36
242	eGFP-S	7.48	4.88	0.89	8.03	5.4
262	eGFP-S	7.481	4.91	0.89	8.03	5.41
282	eGFP-S	7.485	4.76	0.88	8.03	4.85
302	eGFP-S	7.483	4.86	0.88	8.03	4.85
322	eGFP-S	7.48	4.9	0.89	8.03	4.88
342	eGFP-S	7.479	4.87	0.88	8.03	4.85
362	eGFP-S	7.48	4.82	0.89	8.03	4.92
382	eGFP-S	7.471	4.88	0.88	8.02	4.81
	Average:	7.4799474	4.8747368	0.8847368	8.03	5.1789474
	Rel.Std.Dev:	0.0014464	0.0112644	0.0078746	0.0012453	0.0445498
	n	20	20	20	20	20

Table 2. Figures of merit for ruggedness. DNAPac PA200 RS: eGFP-S Ons.

Description	I.D. x L, mm	Particle Size, µM	Cat. No.	Unit
DNAPAC PA200 Nucleic Acid Column	4 x 250	8	**10044-476**	**Each**
DNAPAC PA200RS Nucleic Acid Column	4.6 x 150	4	**10044-906**	**Each**

High Efficiency E. coli Strains for Phage Display Combinatorial Peptide Libraries

Lynne Sheets, Chris Niebauer, and Kristen Terry

ABSTRACT

Phage display technology allows expression of foreign peptides and proteins on the surface of filamentous bacteriophages. This methodology is used to identify peptide ligands for a wide variety of targets by screening for the ability to bind with high affinity and specificity. Phage display has become a cornerstone method to investigate molecular interactions involving protein surfaces.

Library construction using existing technology can generate 3×10^8 recombinants, which is adequate for coverage of the hexapeptide sequence space ($20^6 = 6.4 \times 10^7$). Although random peptide libraries with longer amino acid sequences have been constructed, they are of limited utility because libraries are not sufficiently large to completely explore the additional sequence space.

Other variations of phage display, such as antibody display and cDNA display, incorporate large proteins into the virion. The functional utility of these libraries is also limited by the number of transformants that can be generated.

New strains of *E. coli* engineered for enhanced DNA uptake can improve the transformation efficiency over existing methods by approximately ten-fold ($2\text{-}4 \times 10^{10}$cfu/µg). These improvements can dramatically increase the absolute number of recombinants for challenging applications, significantly reducing the cost to produce and screen phage display libraries of peptides and proteins.

METHODS

The electrocompetent cells were made using a proprietary method of cell preparation developed by Lucigen. This method produces electrocompetent cells that have higher transformation efficiencies than that of cells produced using traditional methods.

The transformation efficiency was tested by transforming 10pg of pUC19 DNA into 25µL of cells. A 1.00mm gap electroporation cuvette was used in a Micro Pulser with settings of 10µF, 600 Ohms, 1800V. Following the pulse, 975µL of Recovery Medium was added to the cuvette and the cells resuspended by pipetting up and down three times.

The cells and Recovery Medium were transferred to a culture tube and placed in a shaking incubator at 250rpm for 1 hour at 37°C. The transformed cells were diluted 1/100 and spread on YT agar plates containing carbenicillin. The plates were incubated overnight at 37 °C.

GENOTYPES

ER2738
F´proA+B+ lacIq Δ(lacZ)M15 zzf::Tn10(TetR)/ fhuA2 glnV Δ(lac-proAB) thi-1 Δ(hsdS-mcrB)5

MC1061 F-
araD139 Δ(araA-leu)7697 Δ(lac)X74 galK16 galE15(GalS) λ- e14- mcrA0 relA1 rpsL150 (StrR) spoT1 mcrB1 hsdR2

SS320 (MC1061F´)
F´[proAB+lacIqlacZΔM15 Tn10 (TetR)]hsdR2 hsdM+ hsdS+ araD139 Δ(ara-leu)7697 Δ(lac) X74 galE15 galK16 rpsL (StrR) mcrA mcrB1

TG1
supE thi-1 Δ(lac-proAB)Δ(mcrB-hsdSM)5 (rK– mK–) F' [traD36 proAB lacIqZΔM15]

Lucigen®

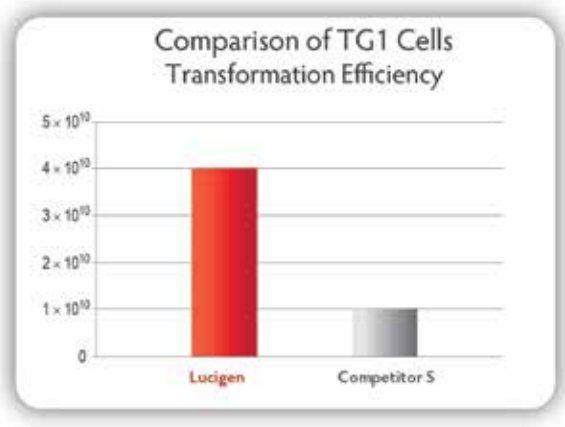
RESULTS

Method Comparison for the Preparation of SS320 Cells

Method of Preparation	Transformation Efficiency
Lucigen	4×10^{10} cfu/µg
Traditional	6×10^{9} cfu/µg

Lucigen SS320 cells were compared with cells prepared with traditional methods.

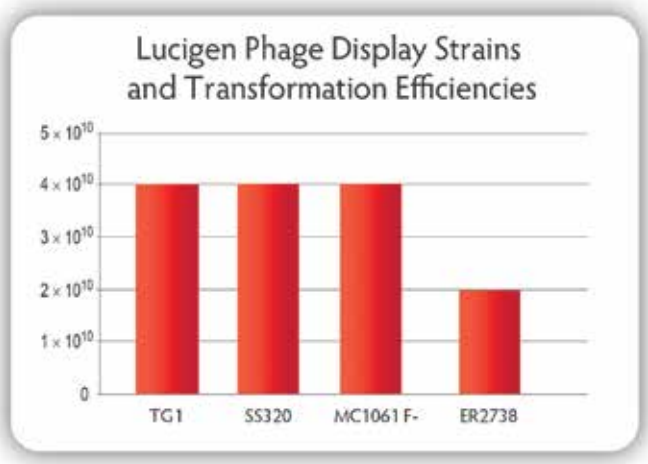
Comparison of Transformation Efficiency of TG1 Cells



Company	Transformation Efficiency
Lucigen	4×10^{10} cfu/µg
Competitor S	1×10^{10} cfu/µg

The Lucigen TG1 cells were compared with cells from competitor "S" using pUC DNA.

Comparison of Phage Display Strains



Cell Line	Transformation Efficiency
TG1	4×10^{10} cfu/µg
SS320	4×10^{10} cfu/µg
MC1061 F-	4×10^{10} cfu/µg
ER2738	2×10^{10} cfu/µg

Transformation efficiency of Lucigen electrocompetent cells for phage display using pUC DNA.

SUMMARY

- The proprietary protocol developed by Lucigen allows greater *E. coli* host strain transformation efficiencies.
- Competent cells produced by Lucigen outperform competitor cells by up to 5-fold.
- Several strains of Lucigen's phage display competent cells are available with exceptional transformation efficiencies (> 2×10^{10}cfu/µg with pUC DNA).

Description	Size	Cat. No.	Unit
E. cloni 10G Chemically Competent Cells	SOLO, 12 Rxn	**89005-078**	**Each**
E. cloni 10G Classic Electrocompetent Cells	Six Pack, 24 Rxn	**89002-684**	**Each**
Endura Chemically Competent Cells	SOLO, 12 Rxn	**71003-032**	**Each**
Endura Electrocompetent Cells	DUO 12 Rxn	**71003-036**	**Each**

Corning® Enhanced Attachment Microcarriers Offer an Ideal Solution for Bioprocess Applications

Hilary Sherman and Mark Rothenberg, Ph.D. Corning Incorporated Life Sciences

INTRODUCTION

The manufacturing of biological therapeutics is a complex and important part of the biopharmaceutical industry. Many biological drugs such as antibodies, hormones, and other factors are produced using engineered cell lines.[1-4] Microcarriers have been used as an alternative to traditional static cultures because of the ability to culture large quantities of adherent cells in a fraction of the space using methods typical of suspension culture. The ability to decrease surface area to volume ratio, increase scalability, and easily separate protein product from cells makes microcarriers an ideal choice for many cell therapies.[5] The new Corning microcarriers offer the same advantages of traditional microcarriers with the added benefits of being sterile and ready-to-use without any prepatory steps (e.g., swelling, washing, etc.).

In this study, Corning Enhanced Attachment Microcarriers are used to effectively culture an M-CSF (macrophage colony stimulating factor) secreting, engineered Chinese Hamster Ovary (CHO) line. The data demonstrates how the Enhanced Attachment Microcarriers are an effective and user-friendly tool compared with an equivalent, commercially available brand.

MATERIALS AND METHODS

Microcarrier Preparation

Corning Enhanced Attachment Microcarriers were transferred to a 150mL storage bottle and reconstituted in sterile cell culture water to a volume of 100mL (36cm^2/mL). Corning Enhanced Attachment Microcarriers are treated with Corning CellBIND® surface, which infuses more oxygen into the plastic for improved cell attachment.

Competitor microcarriers were prepared per manufacturer's recommendations. Briefly, microcarriers were reconstituted in a siliconized glass container in 300mL of phosphate buffered saline (PBS) and swelled overnight at room temperature. After swelling, microcarriers were washed once with PBS and then autoclaved.

Finally, the PBS was removed and the microcarriers were reconstituted in PBS to a concentration of 24mg/mL (105cm^2/mL). Both Corning and competitor microcarriers equivalent to 75cm^2 were transferred to Corning 15mL centrifuge tubes in duplicate. Unused microcarriers were stored at 4°C.

Cell Seeding

After the microcarriers settled, the buffers from each sample were gently aspirated, and each tube of microcarriers was resuspended in IMDM supplemented with 10% FBS containing 3.75 x 10^5 5/9m alpha3-18 cells (ATCC® Cat. No. CRL-10154™). Cells and microcarriers were transferred to Corning 125mL disposable spinner flasks. The volume in each spinner flask was brought up to 30mL by adding additional media. The spinners were stirred at 30rpm for 2 minutes every 30 minutes for 3 hours to allow sufficient time for cell attachment. After the attachment period, an additional 15mL of media was added to each vessel and the stir rate was changed to continuous stirring at 30rpm. Cells were cultured in a 37°C incubator at 5% CO_2 for 4 days. The entire study was repeated three independent times.

M-CSF Production

To evaluate M-CSF production, 1mL of media was collected from each sample prior to harvest on day 4. Samples were centrifuged at 300 x g for 4 minutes to remove debris (cells and microcarriers). The supernatants were stored at -20°C until they were assessed using a human M-CSF ELISA kit.

Nuclei Assessment

Total adherent cells were assessed by collecting 10mL of microcarriers and centrifuging at 300 x g for 6 minutes in order to separate microcarriers from media. A sample from the media was enumerated on the BioProfile® Flex Analyzer (Nova® Biomedical) to assess the number of cells in suspension. One mL of cell lysis buffer was added to microcarriers for less than 1 minute, followed by 3mL of stabilizing buffer. Lysed cells were then separated from microcarriers using a Falcon® 40µM cell strainer with a 50mL centrifuge tube. An additional 2mL PBS rinse was used to wash the cell strainer.

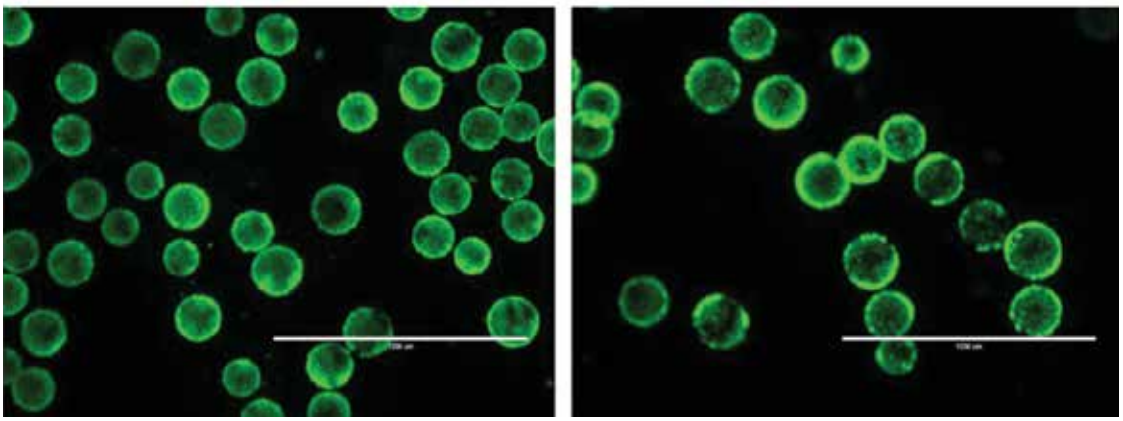


Figure 1. Calcein AM stained 5/9m alpha3- 18 cells on Corning Enhanced Attachment microcarriers (left) and competitor microcarriers (right). Similar confluence and distribution of 5/9m alpha3-18 cells were observed on both microcarriers.

Cells were stained with Hoechst and enumerated using a hemacytometer.

Trypsin Harvesting

To assess the utility of the microcarriers for cell scale-up culture, a trypsin harvest was performed. 10mL of microcarriers from each vessel were centrifuged at 300 x g for 6 minutes in order to separate microcarriers from media. The microcarriers were washed once with 10mL of PBS and then centrifuged again to remove the buffer. 2mL trypsin were added to each sample. The samples were then transferred to wells of a Costar® 6-well plate for monitoring cell dissociation. Once cells began to round up, a P1000 pipettor was used to further detach the cells and 2mL of culture media were added to quench the sample. Cells were separated from microcarriers by using a 40µM cell strainer with a 50mL centrifuge tube. An additional 2mL PBS rinse was performed to wash the 6-well plate and the cell strainer of any additional cells. Cells were then counted on the Nova Bioprofile Flex Analyzer.

RESULTS AND DISCUSSION

5/9m alpha3-18 cells were expanded on microcarriers for 4 days. Similar confluence and cell distribution were confirmed on day 4 by staining cells with Calcein AM (Fig. 1). Paired T-tests indicated that a greater number of cells attached to Corning Enhanced Attachment Microcarriers (Fig. 2) ($P<0.05$) and a greater number of viable cells were recovered with trypsin from the Enhanced Attachment Microcarriers compared to the competitor microcarriers (Fig. 3) ($P<0.001$). Finally, taking into account adherent, as well as any suspension 5/9m alpha3-18 cells, there was no statistical difference in M-CSF production per cell regardless of which microcarrier was used (Fig. 4) ($P>0.05$).

CONCLUSIONS

- Corning® Enhanced Attachment Microcarriers are an ideal choice for use with engineered mammalian cell lines.
- Corning Enhanced Attachment Microcarriers can be used for biomanufacturing and scale-up for biotherapeutic production.
- Higher densities of 5/9m alpha3-18 cells were achieved on Corning Enhanced Attachment Microcarriers as compared to competitor microcarriers.
- Viable 5/9m alpha3-18 cells are easier to detach from Corning Enhanced Attachment Microcarriers as compared with competitor microcarriers.
- 5/9m alpha3-18 cells produced equivalent M-CSF/cell when cultured in both microcarrier types that were tested.

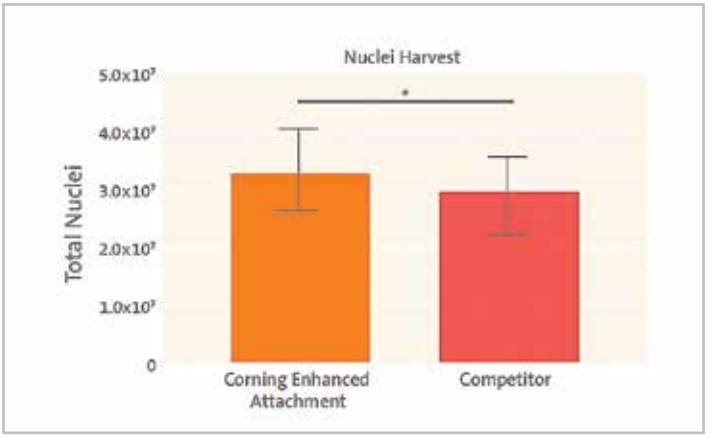


Figure 2. Total 5/9m alpha3-18 nuclei from 75 cm² of Enhanced Attachment and competitor microcarriers. A paired t-test shows that statistically more adherent nuclei were collected from 75cm² of Enhanced Attachment Microcarriers compared to 75cm² of competitor microcarriers. Unpaired t-test* P<0.05 (n=6).

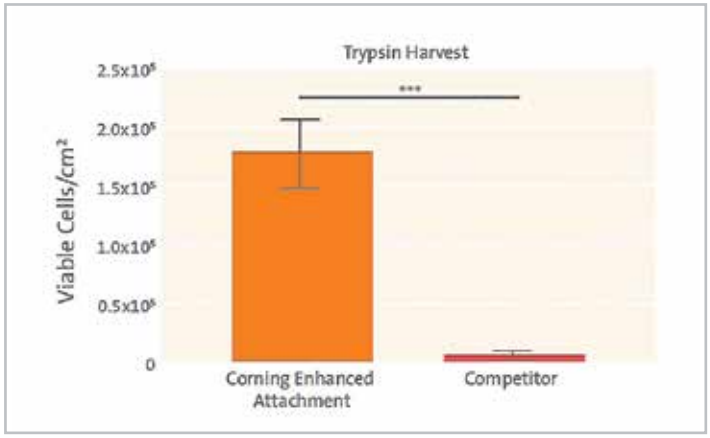


Figure 3. Adherent 5/9m alpha3-18 cell densities harvested from 75cm² of Enhanced Attachment and competitor microcarriers. A paired t-test shows that statistically more adherent 5/9m alpha3-18 cells were harvested from 75cm² of Enhanced Attachment Microcarriers as compared to 75cm² of competitor microcarriers. Unpaired t-test*** P<0.0001 (n=6).

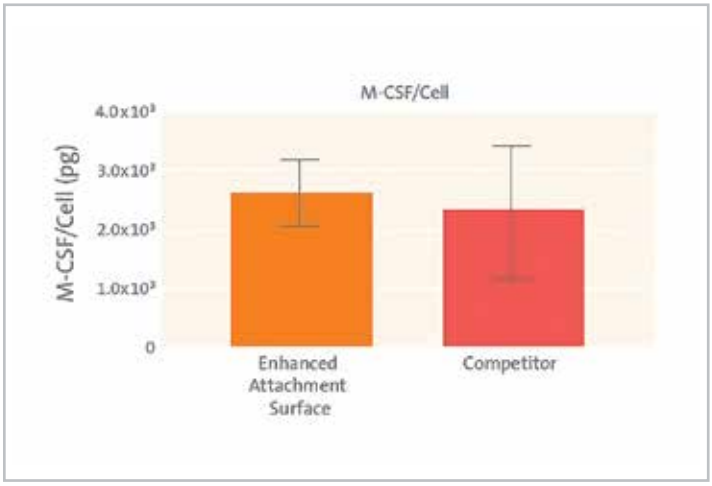


Figure 4. M-CSF production from 5/9m alpha3-18 cells cultured on Corning Enhanced Attachment and competitor microcarriers. A Paired t-test shows no statistical difference in M-CSF production/cell between Enhanced Attachment Microcarriers and competitor microcarriers.
*Calculated concentration includes cells found in suspension as well as cells attached to microcarriers. Unpaired t-test P>0.05 (n=6).

References

1. *Jostock T, Knopf HP. Mammalian stable expression of biotherapeutics.* Methods Mol Biol. *2012;899:227-38. doi: 10.1007/978-1-61779-921- 1_15.*
2. *Drugmand JC, Schneider YJ, Agathos SN. Insect cells as factories for biomanufacturing.* Biotechnol Adv. *2012 Sep-Oct;30(5):1140-57.*
3. *Zhang J. Mammalian cell culture for biopharmaceutical production. In: Baltz RH, Davies JE, Demain AL, eds.* Manual of Industrial Microbiology and Biotechnology, Third Edition. *Washington, DC: American Society of Microbiology; 2010:157-178.*
4. *Palomares LA, Estrada-Mondaca S, Ramirez OT. Production of recombinant proteins: challenges and solutions. In: Balbas P, Lorence A, eds.* Methods in Molecular Biology, *Vol. 267: Recombinant Gene Expression: Reviews and Protocols, Second Edition. Totowa, NJ: Humana Press Inc. 2004:15-51.*
5. *Chen AK, Reuveny S, Oh SK. Application of human mesenchymal and pluripotent stem cell microcarrier cultures in cellular therapy: Achievements and future direction.* Biotechnol Adv. *2013 Mar 24.pii: S0734-9750(13)00065-7. doi: 10.1016/j.biotechadv.2013.03.006. (Epub ahead of print).*

Description	Size	Cat. No.	Unit
Dulbecco's Phosphate-Buffered Saline (DPBS), 1X without Calcium and Magnesium	1 L	**45000-436**	**Pk. 6**
Enhanced Attachment Microcarriers	10 g	**30617-554**	**Each**
Fetal Bovine Serum (FBS), USDA Approved	500 mL	**45000-734**	**Each**
Iscove's Modification of DMEM with L-glutamine and 25 mM HEPES without ß-thioglycerol and ß-mercaptoethanol	1 L	**45000-368**	**Pk. 6**
Trypsin EDTA 1X 0.05% Trypsin/0.53 mM EDTA in HBSS without sodium bicarbonate, calcium, and magnesium Porcine Parvovirus Tested	500 mL	**45000-662**	**Pk. 6**
Water, Cell Culture Grade, Tested to USP Sterile Water for Injection Specifications	1 L	**45001-042**	**Pk. 6**

Growth Comparison Studies Between Fetal Bovine Serum and Other Serum Products

GE Healthcare HyClone™ Serum Lab

Distributor
GE Healthcare

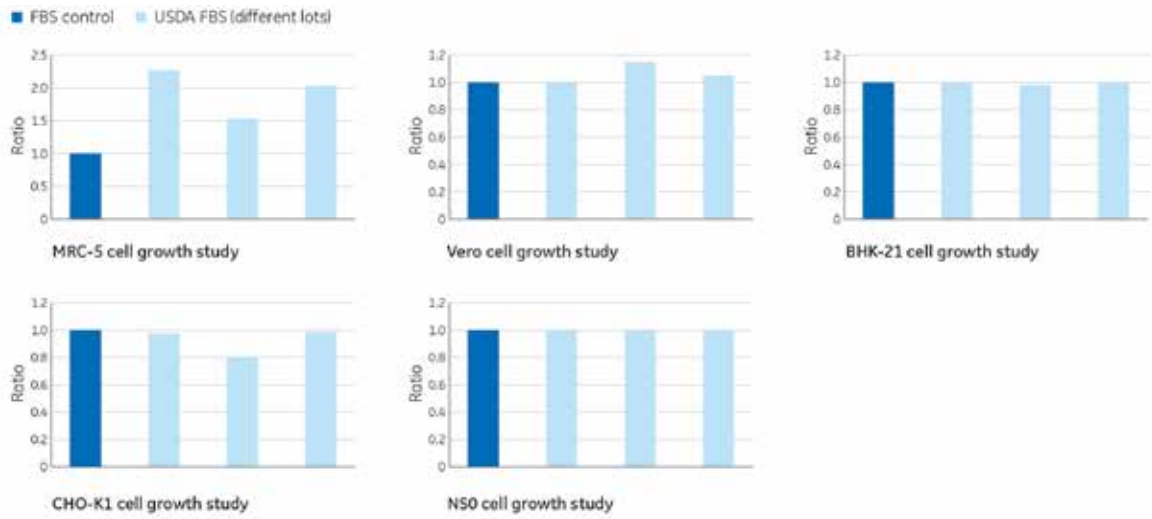
Fetal bovine serum (FBS) is the serum of choice for cell and tissue culture. While FBS is an excellent product for many applications, it is subject to shortages and consequently price fluctuations and availability difficulties. As an alternative, HyClone™ Bovine Calf Serum was introduced to the market in various forms. The calf serum products provide advantages in availability, price, and consistency in performance. In 1984, Iron-Supplemented Bovine Calf Serum was the first HyClone serum product to be introduced. Since then, HyClone has introduced other supplemented and processed calf serum products including Cosmic Calf™ Serum, FetalClone™, and, most recently, Bovine Growth Serum (BGS). To demonstrate the ability of these products to perform as well as FBS in many applications, we provide data comparing FBS to these products along with comparisons of non-US sourced FBS to US sourced FBS.

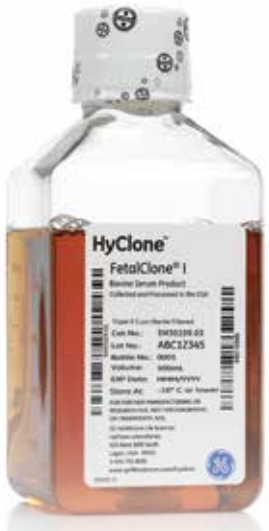
The following growth promotion studies were performed in the Hyclone R&D department. Our non-US sourced FBS, as well as our fetal bovine serum replacements were compared with a US sourced FBS control for their ability to promote culture growth in a variety of popular cell lines. All cultures were grown in T-25 cell culture flasks using 10mL of appropriate medium, which was supplemented with 10% serum and checked daily for confluency. Performance was normalized to the FBS control by dividing the cell count from each FBS alternative condition by the cell count from the FBS control. The resulting ratios are presented as relative yields in each study. The ratio, or relative yield, of FBS is always 1.0. Conditions that produce more cells than the control have values greater than 1.0.

Growth Comparison Studies

US Sourced FBS Controls vs USDA*-Tested Central American Sourced FBS

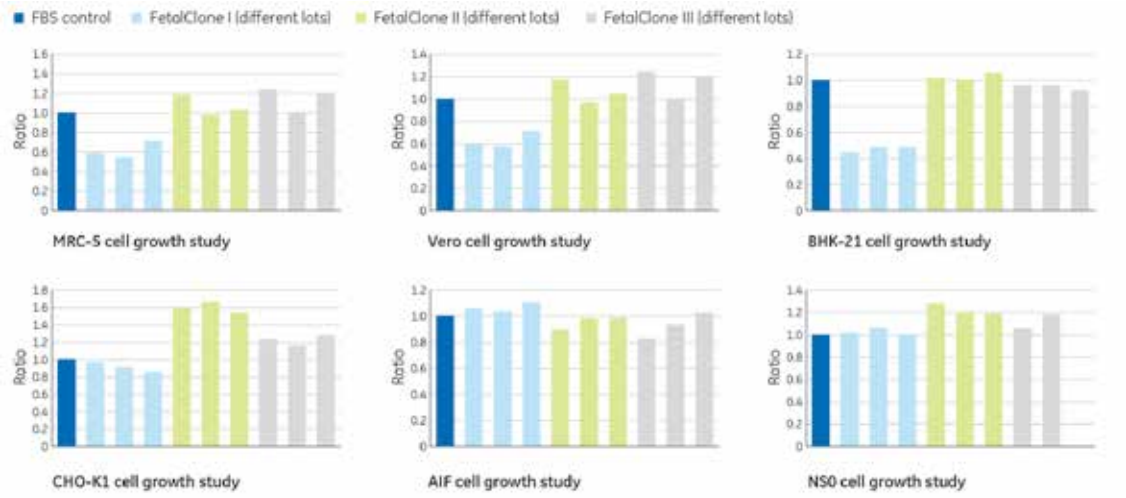
*United States Department of Agriculture



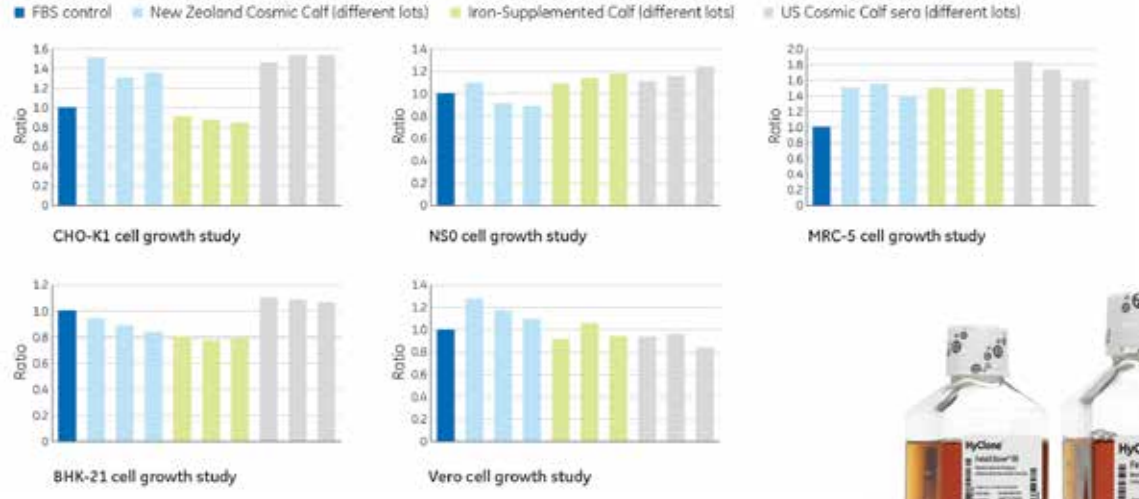


Please contact your VWR Sales Representative for help in selecting the appropriate product for your applications and for samples.

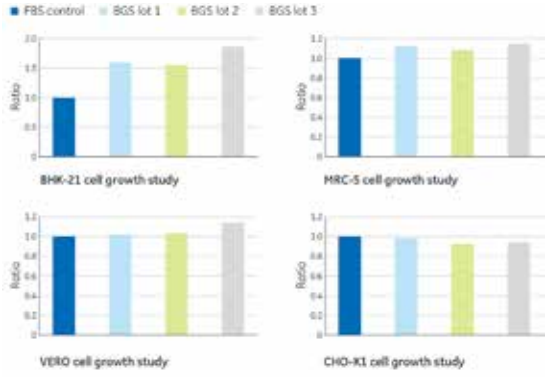
US sourced FBS control vs FetalClone I, II, III



US Sourced FBS Control vs New Zealand Cosmic Calf, Iron-Supplemented Calf, and US Cosmic Calf Sera



US Sourced FBS Control vs Bovine Growth Serum



Description	Cat. No.	Unit
FBS, Characterized (US)	16777-014	Each
Calf Serum Iron Supplemented (US)	16777-022	Each
Cosmic Calf Serum (US)	16777-244	Each
Cosmic Calf Serum (NZ)	82024-636	Each
FetalClone I	16777-232	Each
FetalClone II	16777-236	Each
FetalClone III	16777-240	Each

* All sizes listed here are 500mL

Screening Reactive Oxygen Species (ROS) on iQue® Screener

IntelliCyt Corporation, Albuquerque, New Mexico, USA • Enzo Life Sciences, Farmingdale, New York, USA

SUMMARY

The iQue Screener was used to simultaneously detect levels of ROS and superoxide in multiple cell lines.

- Both Total ROS (non-superoxide) and superoxide were detected simultaneously.
- Two related endpoints were easily discriminated by proper indicator selections and confirmed by differential inhibition.
- Easy-to-use ForeCyt™ provides a friendly screening-centric interface to create assay protocols and analyze results.

PROBLEM

Reactive oxygen species (ROS) is a group of highly unstable molecules, including H_2O_2, NO, and O_2, that are generated in situ from various stressors. At least one ROS in situ molecule (NO) acts as a signaling molecule, migrating across proximal cell membranes to activate guanylyl cyclase and causing smooth muscle relaxation. At higher concentrations, ROS cause oxidative stress and are destructive to lipids, DNA, and proteins. Excessive amounts have been linked with numerous diseases such as cancer, cardiovascular disease, and hearing loss. General aging effects are also thought to be the result of ROS. There are many control mechanisms in play to limit the damage ROS would otherwise cause, including enzymes and vitamins. Early, rapid, and easy identification of compounds that cause increases in ROS would be a valuable component to a drug discovery and development program.

RESULTS

Total ROS and superoxide were simultaneously measured in two different cell lines using the ROS-ID™ Total ROS/ Superoxide detection kit from Enzo Life Sciences. T-cell derived Jurkat cells and HeLa cells both have previously been shown to produce ROS in abundance following stimulation with pyocyanin. Both cell lines were treated with pyocyanin to stimulate ROS and superoxide generation, and with N-acetyl-cysteine (NAC) to inhibit ROS. 5mM NAC inhibited about 90% of total ROS in Jurkat cells treated with 100µM pyocyanin (Figure 1) and 50% in HeLa cells, but only about 20% of superoxide in Jurkat cells and 10% in HeLa cells. This differential inhibition supports the notion that what is detected by the two dyes is different.

THE ENZO/INTELLICYT SOLUTION

Enzo's ROS-ID Total ROS/Superoxide detection kit enables the simultaneous measurement of ROS and superoxides. Combined with Intellicyt's iQue screener, the ROS-ID Total ROS/ Superoxide detection kit allows high-throughput screening of factors involved in oxidative stress. The iQue analyzes individual cells for six parameters. In this application, every sampled cell is analyzed for Total ROS and for superoxide, leaving two fluorescent channels unused. When combined with immunophenotyping, a mixed population like PBMCs could be treated and analyzed for differential alterations to their oxidative states.

The iQue enables assay miniaturization, with as little as 1µL input volume. This means using fewer cells in each analysis, thus opening the door to using primary or stem cells for ROS/SO screening.

Description	Cat. No.	Unit
ROS-ID Total ROS/Superoxide Detection Kit	89165-882	Each

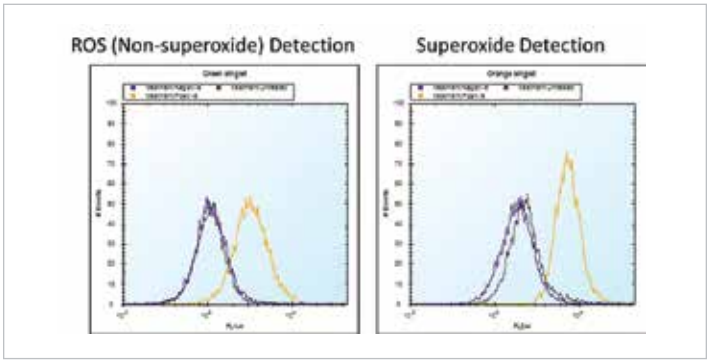


Figure 1. Histogram overlay of control (black), activated (orange), and inhibitor pre-treated (purple) Jurkat cells used in ROS detection. Treated cells were activated for 30 minutes with 200µM pyocyanin after 30 minutes of no treatment or 5mM N-acetyl-cysteine (NAC) pretreatment. Dyes were added after treatments 30 minutes before reading on iQue. HeLa cells responded similarly to the Jurkat cells when treatments were carried out in the presence of Accutase®.

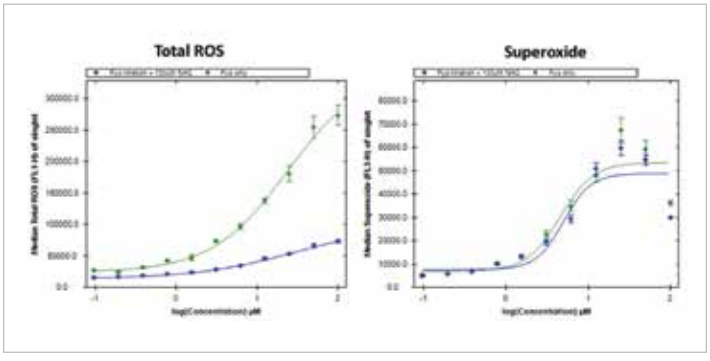
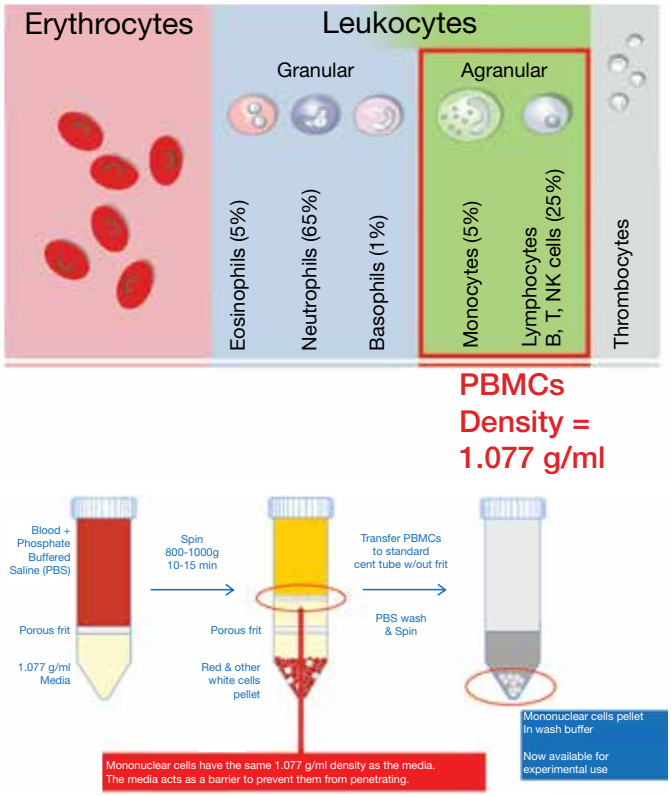


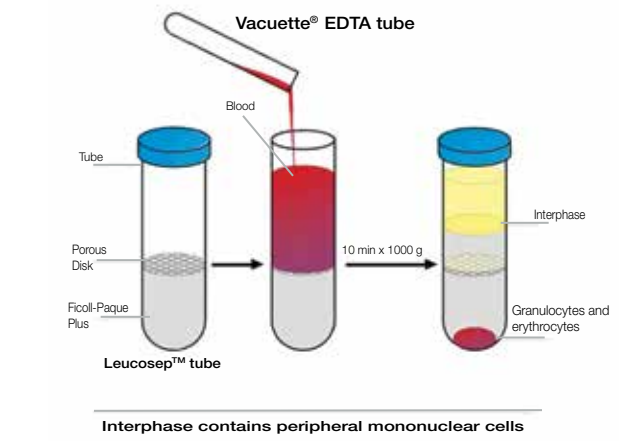
Figure 2. Dose responses of pyocyanin in generating Total ROS or superoxide in HeLa cells, with or without pretreatment with 5mM NAC. Total ROS signal is mostly inhibited by 5mM NAC, whereas superoxide is not. Data are based on triplicate +/- S.D.

SEPARATIONS:

Enrichment of PBMCs from Whole Blood

When conducting density gradient centrifugal separation of lymphocytes and monocytes (PBMCs) from peripheral blood, maintaining a separate and distinct layer between the density gradient media and peripheral blood sample is critical to obtain optimal separation and yield. Preserving the separation integrity of these layer components can present challenges, especially in a demanding workload regimen when multiple specimen separations are being performed, and the required layering steps typically necessitate a diligent effort over time.





Greiner Bio-One Leucosep™ Tubes are designed to maximize the effectiveness of the separation of lymphocytes and monocytes (PBMCs) from whole blood and bone marrow in rapid and easy fashion, while reducing handling of blood specimens. Unique to Leucosep is a porous barrier made of high quality polyethylene, firmly positioned within the polypropylene tube, under which the density gradient media layer is contained to maintain a distinct separation from the peripheral blood specimen.

The resultant Leucosep product enables a rapid pour of the blood sample into the separation tube, eliminating risk of undesired sample/separation media mixing, as well as considerable time-savings to perform the otherwise required overlay/underlay steps. Following centrifugation, the interphase above the fritted disc containing the PBMC fraction may simply be poured out, enabling additional time-saving along with minimal risk of re-contamination with red blood cells during the recovery process.

Leucosep are available in two sizes and may be used in combination with all common separation media for PBMC separation. For maximum convenience, Leucosep tubes are also available pre-filled with Leucosep separation medium, buoyant density 1.077g/mL, which yields excellent separation results.

Instruction Manual Leucosep™ Preparation

Warm up separation medium to room temperature (RT) while protecting it from light.

Fill the Leucosep tube with separation medium: 3mL when using 12mL tubes; 15mL when using 50mL tubes.

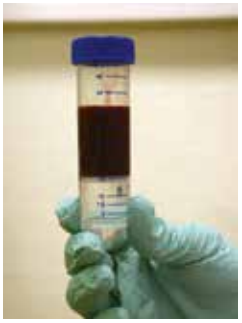
Close the tubes containing the separation medium with the screw cap and centrifugate for 30 sec. at 1000 x g and RT. The separation medium is now located below the porous barrier.

When using tubes that are prefilled with separation medium,the aforementioned steps can be disregarded. Simply warm up the tubes to RT.

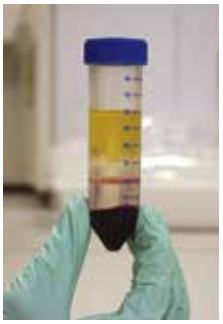
The tubes are now ready for filling with anticoagulated blood or bone marrow aspirate. Dilution of the sample material with balanced salt solution is not implicitly necessary, but it can help to improve the result of the separation. For blood, a dilution ratio of 1:2 is recommended, and for bone marrow a ratio of 1:4 is recommended.

greiner bio-one

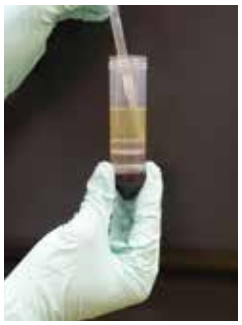
Filling with sample material



Before centrifugation



After centrifugation



Harvest with a Pasteur pipette or by decanting into another centrifuge tube

Typical Results:

Vitality	Viable cells in %	95 ± 5
Cell yield	Lymphocytes in % of original number	60 ± 20
Composition of enriched cell fraction	Mononuclear cells in % Granulocytes in % Erythocytes in %	95 ± 5 5 ± 5 < 1
Composition of lymphocite fraction	T cells % B cells% NK cells %	83 ± 3 6 ± 3 11 ± 2

Two Different Sizes

50 ml conical bottom centrifuge tube
- for larger blood volumes

Porous frit at 15 ml graduation

10 ml round bottom centrifuge tube
- for smaller blood volumes

Porous frit at 3 ml graduation

Procedure

1) Pour the anticoagulated sample material (blood or bone marrow aspirate, diluted with balanced salt solution if necessary) directly from the blood sampling tube carefully into the Leucosep tube: 3–8mL of sample material when using 12mL tubes; 15–30mL of sample material when using 50mL tubes.
2) Centrifuge 10 minutes at 1000 x g and RT or 15 minutes at 800 x g and RT in a swinging bucket rotor. Switch off the brakes of the centrifuge.
3) After centrifugation, collect and discard the plasma layer. The sequence of layers occurs as follows (seen from top to bottom):
   a) Plasma
   b) Enriched cell fraction (interphase)
   c) Separation medium fraction (up to a minimum remnant of 5 to 10mm above the interphase helps to prevent contamination of the enriched cells with platelets)
   d) Porous barrier
   e) Separation medium consisting of lymphocytes/PBMCs
   f) Pellet (erythrocytes and granulocytes).
4) Harvest the enriched cell fraction (lymphocytes/PBMCs) by means of a Pasteur pipette or by pouring the supernatant above the porous barrier from the Leucosep tube into another centrifugation tube. The porous barrier effectively avoids recontamination with pelleted erythrocytes and granulocytes.
5) Wash the enriched cell fraction (lymphocytes/PBMCs) with 10mL of phosphate-buffered saline (PBS), subsequently centrifugate for 10 minutes at 250 x g.
6) Repeat washing step twice, re-suspend the cell pellet with 5mL of PBS.

Caution

Handle all biological samples and blood collection lancets, needles, and blood collection sets in accordance with the policies and procedures of your facility. In case of any exposure or contamination with blood or other biological samples, (e.g., accidental puncture injury) initiate appropriate medical treatment as such material has to be considered potentially infective with HBV, HCV (hepatitis), HIV (AIDS), or other infective agents.

Description	Cat. No.	Unit
12 mL Leucosep® Tube w/Porous Barrier at 3 mL graduation, PP		
Aseptic, Round Bottom, Pre-filled w/1.077 g/mL PBMC Separation Medium	**89218-662**	**Pk. 50**
Sterile, Round Bottom, Un-filled	**89048-934**	**Pk. 50**
50 mL Leucosep Tube w/Porous Barrier at 15 mL graduation, PP		
Aseptic, Conical Bottom, Pre-filled w/1.077 g/mL PBMC Separation Medium	**89136-192**	**Pk. 25**
Sterile, Conical Bottom, Un-filled	**89048-938**	**Pk. 25**

Ghost Dye™ Viability Dyes

A Powerful Tool for Flow Cytometric Immunophenotyping Experiments

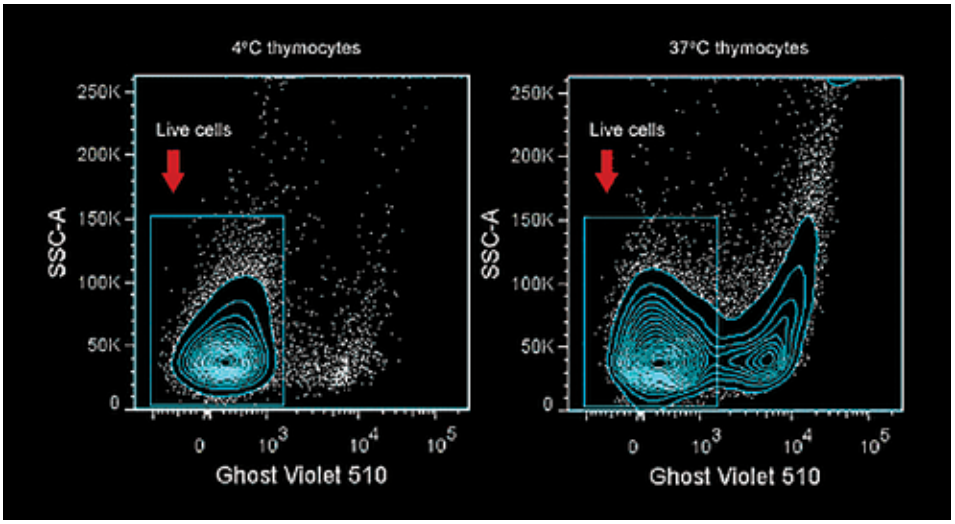


Figure 1. Mouse thymocytes were incubated overnight at 4°C (left) or 37°C (right) and stained with Ghost Dye Violet 510. Viable gate is indicated. In the right plot, heat-treated samples include a dead cell population that stains significantly brighter than live cells and can be effectively excluded from further data analysis. The smaller dead cell population from the 4°C sample is also clearly separated and easily gated out.

Tonbo Bioscience's Ghost Dyes are bright, amine-reactive, fixable viability dyes. They allow for clear cell discrimination between live and dead cells for more precise flow cytometric analysis, and are especially useful with intracellular staining protocols.

Ghost Dyes bind irreversibly to amine groups and are resistant to subsequent washing, fixation, and permeabilization, leaving behind a bright and stable fluorescent signal. On live cells, this class of dye is only able to bind to surface amines. Dead cells with compromised membranes allow the dye to permeate and bind amine groups of intracellular proteins within the cytoplasm. This results in a fluorescent signal much brighter than that from live cells, which are impermeable to the dye (Figure 1).

Ghost Dyes can be used in situations where DNA dyes like 7-AAD and Propidium Iodide (PI) are not recommended, such as with protocols that require fixation or fixation and permeabilization steps. Under those conditions, cell membranes are compromised and nuclear dyes that bind reversibly can leak out resulting in substantial signal loss. Cells labeled with a Ghost Dye can also be cryopreserved for later use without loss of fluorescence intensity. Note that no dead cell exclusion dye can be used with cells that are already fixed.

Flow core facility managers are encouraging users to include a dead cell exclusion dye in every experiment to ensure quality of the data and the accuracy of downstream analysis. It is particularly important when working with rare populations or sensitive samples (for example, tissue that needs to be processed or fragile cells sensitive to manipulation). Even in routine experiments, excluding dead cells can reduce false positives and increase reproducibility, as dead cells are known to be stained non-specifically by antibodies and other reagents. Figure 2 demonstrates that more precise data can result when dead cells are excluded with higher accuracy.

Tonbo offers a selection of Ghost Dye fixable viability dyes that can be excited with a variety of lasers for flexibility in experimental design. Dyes are provided ready-to-use in solution and should be stored at -20°C. Heat-treated cells stained with the appropriate Ghost Dye can be used as a compensation control. Alternatively, amine-functionalized microspheres can be substituted for cellular controls.

Ghost Dye UV 450 is excited by the UV (355nm) laser line and has a peak emission of 450nm that can be detected using 450/50 band pass filters commonly used for detection of DAPI, Hoechst 33258, etc.

TONBO biosciences

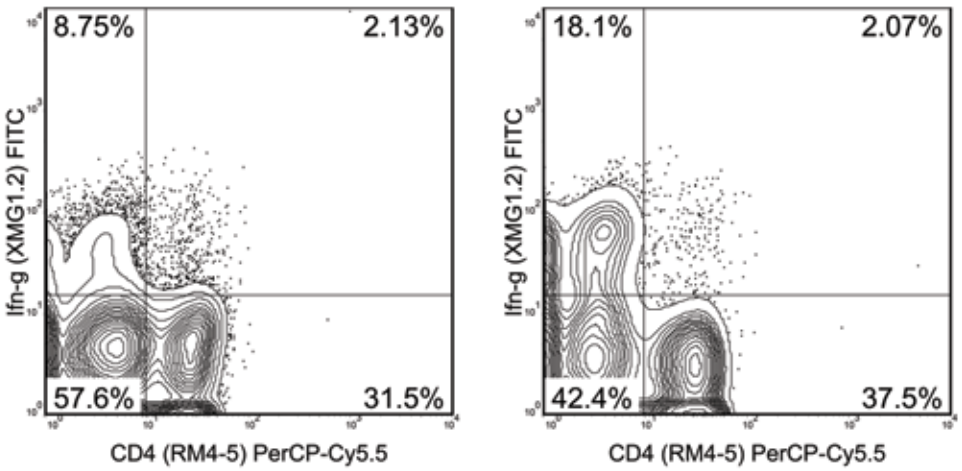


Figure 2. Mouse splenocytes were stimulated with PMA + ionomycin and stained with anti-CD4 and anti-IFN-γ antibodies, with or without a Ghost Dye. In the left plot, no viability dye was included, but in the right plot, dead cells were more accurately excluded. The resulting data analysis shows the improved resolution of populations that is gained when dead cells are excluded from analysis.

Ghost Dye Violet 450 is excited by the violet (405nm) laser line and has a peak emission of 450nm that can be detected using 440/40 or 440/50 band pass filters commonly used for detection of violetFluor™ 450, Pacific Blue®, BD Horizon™ V450 or eFluor® 450.

Ghost Dye Violet 510 is excited by the violet (405nm) laser line and has a peak emission of 510nm that can be detected using a 525/50 band pass filter commonly used for detection of AmCyan.

Ghost Dye Red 710 is excited by the red (633-647nm) laser line and has a peak emission of 710nm that can be detected using the recommended 710/50 band pass filter commonly used for detection of Alexa Fluor® 700.

Ghost Dye Red 780 is excited by the red (633nm) laser line and has a peak emission of 780nm that can be detected using a 780/60 band pass filter commonly used for detection of APC-Cy7.

Excitation Laser (nm)	Peak Emission (nm)	Size	Cat. No.	Unit
Ghost Dye™ UV 450				
355	450	100 Tests	**10274-082**	**Each**
355	450	500 Tests	**10274-084**	**Each**
Ghost Dye™ Violet 450				
405	450	100 Tests	**10140-978**	**Each**
405	450	500 Tests	**10141-462**	**Each**
Ghost Dye™ Violet 510				
405	510	100 Tests	**10140-892**	**Each**
405	510	500 Tests	**10140-980**	**Each**
Ghost Dye™ Red 710				
633-647	710	100 Tests	**10002-364**	**Each**
633-647	710	500 Tests	**10002-098**	**Each**
Ghost Dye™ Red 780				
633	780	100 Tests	**10140-888**	**Each**
633	780	500 Tests	**10140-890**	**Each**

Select publications citing Tonbo Ghost Dyes:

Lee AR, Mao C, Vo H, Gao W and Zhong X. 2015. Fluorescence tagging and inducible depletion of PD-L2-expressing B-1 B cells *in vivo*. *Ann NY Acad Sci.* 1362(1): 77-85.

Milner JD, Vogel TP, Forbes L, Ma CA, Stray-Pedersen A, Niemela JE, Lyons JJ, Engelhardt KR, Zhang Y, Topcaqic N et al. 2015. Early-onset lymphoproliferation and autoimmunity caused by germline STAT3 gain-of-function mutations. *Blood.* 125 (4): 591-599.

Vo H, Chiu J, Allaimo D, Mao C, Wang Y, Gong Y, Ow H, Porter T and Zhong X. 2014. *Immun, Inflamm & Dis.* 2(4): 254-261.

Watkin LB, Jessen B, Wiszniewski W, Vece TJ, Jan M, Sha Y, Thamsen M, Santos-Cortez RL et al. 2015. COPA mutations impair ER-Golgi transport and cause hereditary autoimmune-mediated lung disease and arthritis. *Nat Genet.* 47: 654-660.

Wood S, Feng J, Chung J, Radojcic V, Sandy-Sloat AR, Friedman A, Shelton A, Yan M, Siebel CW, Bishop DK and Maillard I. 2015. *J Immunol.* 194(6): 2899-2908.

PeproGrow Human Embryonic Stem Cell Media

Maintenance Media for hESCs and hiPSCs

PeproTech Technical Support Team

PeproGrow hESC is a serum- and phenol red-free medium of a complete, chemically-defined formulation designed for feeder-free maintenance and expansion of both human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) using Corning Matrigel® as a surface-coating matrix. This medium is intended for the culturing of hESCs and hiPSCs in the undifferentiated, pluripotent state (SSEA4+/Oct4+) and demonstrates less than 15% spontaneous differentiation as indicated by flow cytometry. The proprietary formulation of the medium includes relevant growth factors, such as FGF2 (FGF-basic), but does not contain the insulin found in the majority of other hESC/hiPSC media currently available on the market. PeproGrow hESC, which was designed and developed by PeproTech in collaboration with the Stem Cell Training Course at Rutgers University, is supplied as a 100mL or 500mL bottle of basal medium and a separate, lyophilized growth factor component.

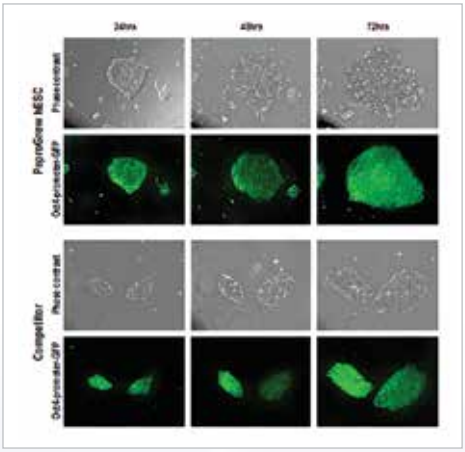
Specifications

Sterility	Negative
pH	7.35-7.40
Osmolality	340-350mOsm

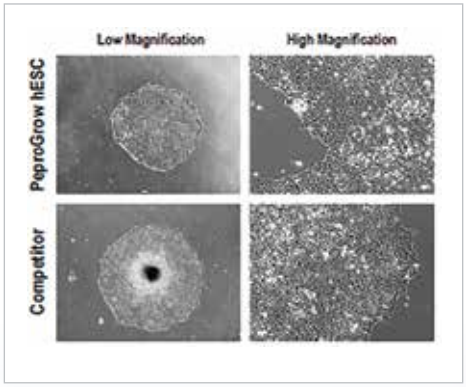
Benefits

- Phenol red-free
- Insulin-free
- Complete & chemically-defined
- High plating efficiency
- High quality recombinant growth factors

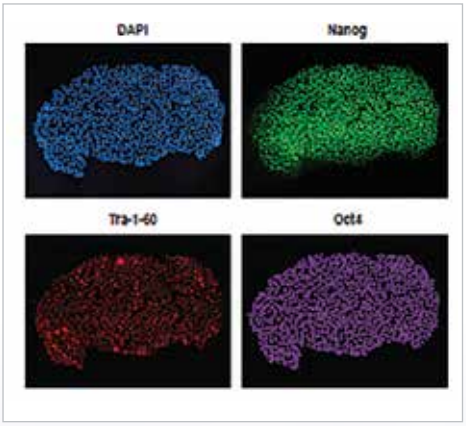
Description	Size	Cat. No.	Unit
PeproGrow hESC	100 mL	**10771-872**	**Each**
PeproGrow hESC	500 mL	**10771-874**	**Each**



The growth of H1 hESCs expressing turboGFP-NEO under the control of the Oct4 promoter. H1 hESCs modified by lentivirus to contain the Oct4 promoter driving turboGFP, were cultured in PeproGrow hESC medium, were passaged using dispase, and plated in PeproGrow hESC medium containing 2µM Y-27632 onto Corning Matrigel® coated 6-well dishes. Cultures were fed daily and photographed 24, 48, and 72 hours post split. Green fluorescence represents maintenance of pluripotency as indicated by Oct4 promoter activity. PeproGrow hESC maintains cells in the pluripotent state like the competitor media and allows the cells to plate out better (faster confluency).



Cell Morphology of hESCs cultured in PeproGrow hESC medium. This medium contains a unique blend of cytokines that at first may alter the cellular or colony appearance, making them more flat and elastic; however, this morphological change appears less dramatic as the cell density increases, or over several passages. Stem cells will form standard tight circular colonies (left), and have expected morphology with large nucleus and small cytoplasm (right).



Immunostaining of iPSCs cultured in PeproGrow hESC medium. Cells were plated on 24-well dishes, paraformaldehyde fixed, and then stained with DAPI and other indicated antibodies, which indicate cell pluripotency.

Irradiated Sterile Environmental Monitoring Plates

HARDY DIAGNOSTICS
A Culture of Service™

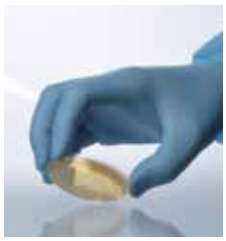
Environmental monitoring is such a commonplace practice, monotonous and mundane, that many often get complacent over the importance of proper quality assurance practices. Nonetheless, researchers should never lose sight of the overwhelming importance of proper monitoring of lab surfaces, as a simple lapse in judgment can ruin not only a lab's current work, but their reputation.

In environmental testing, it is not simply the quality of the equipment and media that determines clear and concise results, but also proper procedure and technique. Here we outline the proper usage of TapTight™ sterile contact plates and SterEM™ sterile environmental monitoring plates.

Prior to use, check the bags (irradiated plates come triple bagged) and plates for condensation from storage. If condensation is present, remove the innermost bag in a sterile environment and allow to dry for ten to fifteen minutes prior to use. Proper gowning and glove procedures must be followed with environmental monitoring to avoid cross contamination.

When using contact plates, you must be especially diligent that you are testing only surfaces and not the falling sediments in the air. When you remove the lid of the contact plate, do not invert the lid once it is removed in order to avoid exposure to falling sediment. Sample the surface by firmly pressing the agar against the test area using the thumb and second finger to hold the plate while using the index finger to press the plate firmly and evenly against the base. Place equal pressure on each sample. Do not twist the plate or move it across a surface, as this spreads contaminates across the agar surface. A rolling motion can be used when curved surfaces or edges must be sampled. TapTight contact plates contain a grid, allowing assays on specific areas of the grid. Once you are done with your surface sampling, take the contact plate and place the lid gently on top. With one finger, you can press down on the TapTight friction lid to secure the lid.

In addition to surface sampling, air sediment sampling can be done with SterEM settling plates. Like the TapTight plates, the SterEM plates are also irradiated. While the TapTight plates are validated for sterility assurance level of 10^{-6}, SterEM plates are validated at 10^{-5}. To use SterEM, simply unpackage the triple bagged plates in a clean room setting, remove the lid and lay the plate on a paper towel with the agar exposed to the air. With the proper tools and due diligence, you can keep contaminates from ruining your delicate experiments.



Description	Sampling Type	Fill Volume, mL	Plate type	Storage Temp	Cat. No.	Unit
D/E Neutralizing Agar	Surface/Fingertip	15	60mm; Contact	2-8°C	**10142-498**	**Pk. 10**
Sabouraud Dextrose (Sabdex) Agar	Air	26	100mm; Regular	2-8°C	**10142-504**	**Pk. 10**
Sabouraud Dextrose (Sabdex) Agar with Lecithin and Tween® 80	Surface/Fingertip	15	60mm; Contact	2-8°C	**10142-448**	**Pk. 10**
Tryptic Soy Agar (TSA)	Air	26	100mm; Regular	2-8°C	**10142-500**	**Pk. 10**
Tryptic Soy Agar (TSA)	Air	34	100mm; Regular	2-8°C	**10142-502**	**Pk. 10**
Tryptic Soy Agar (TSA), Red Plate	Air	34	100mm; Regular	2-8°C	**10146-802**	**Pk. 10**
Tryptic Soy Agar (TSA, Soybean Casein Digest Agar) with Lecithin and Tween® 80, HPV Impermeable Mylar Bag	Surface/Fingertip	15	60mm; Contact	15-30°C	**10142-324**	**Pk. 10**
Tryptic Soy Agar (TSA, Soybean Casein Digest Agar) with Lecithin and Tween® 80, Red Plate, HPV Impermeable Mylar Bag	Surface/Fingertip	15	60mm; Contact	15-30°C	**10146-962**	**Pk. 10**

How Do I Know if I'm Using the Right Biological Safety Cabinet for My Process?

Brian Garrett, LEED Green Associate, Product Manager

Whether you're working with Ebola, cancer cells, or DNA samples, how can you be sure that your lab contains the right kind of equipment to protect yourself, your labmates, and your precious samples? Different biosafety enclosures are built for very different purposes; using the wrong one could contaminate your samples or, worse, put you in harm's way.

Know Your Biosafety Level

The Biosafety Levels (BSLs) of various laboratories are designated by the Centers for Disease Control and Prevention (CDC) and take into account the agents (microbes) used, the processes within the lab, and the lab's design criteria. The levels vary from BSL 1 to 4, with BSL 4 representing the highest risk. Even Class I biological safety cabinets (BSCs) offer proper protection to their user for BSL 1 through 3. Unfortunately, Class I BSCs don't protect the materials inside the BSC from contamination.

Class II BSCs are also recommended for various BSL 1, 2 and 3 applications. Only in rare cases are Class III, glove box-type enclosures required. Even in Biosafety Level 4 applications, a combination of environmental and personnel protection, such as biosafety suits, can be used so that procedures can be conducted in Class II BSCs. Consult your safety officer regarding your biosafety level and contact your VWR rep if you are unsure of which enclosure is right for your application.

Protecting Your Samples

Consider how vulnerable your samples are to contamination from room air before selecting a Class I enclosure for your procedure. Penicillin has already been discovered, so unless you're looking to reproduce Louis Pasteur's famous accidental revelation, work in a Class II BSC when you have biological samples that require protection from the environment.

Using Chemicals in a BSC

Biological hazards and chemical hazards are very different because chemical hazards can be diluted to safe levels, but biological hazards can proliferate from very small quantities. A microscopic sample of the Ebola virus can do a great deal more harm than a similar quantity of chemicals.

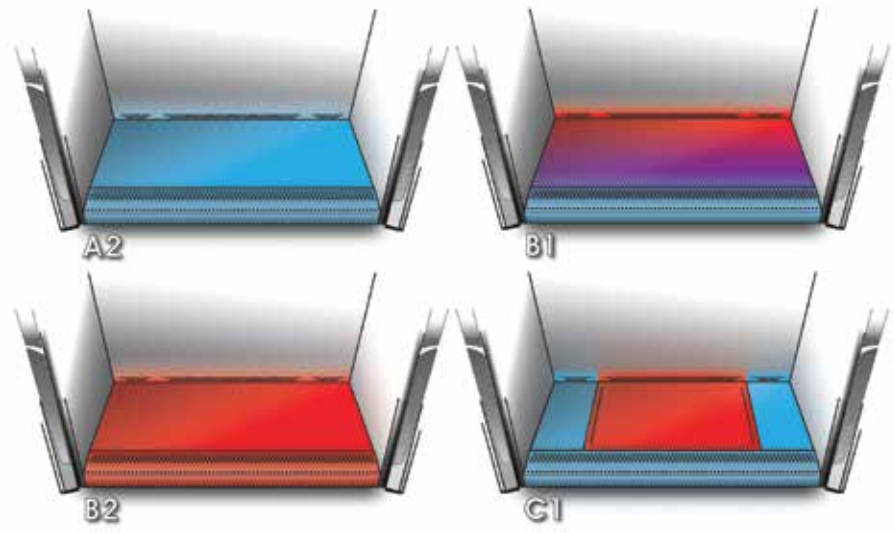


Figure 1. From the operators' vantage point – selecting, training, and use of each of the Class II BSCs is slightly different. Red zones indicate areas where airflow is single-pass and allows for handling hazardous chemistry. Blue zones are those where the airflow is recirculating and cannot be used for hazardous chemical work. The B1 and C1 are the only two that function with both recirculating air and single-pass air; however, the C1's clearly defined work area takes the guess work out of where to work safely (see the zone of Purple on the B1).

* Class II Type C BSCs are pending final designation at the time this article was written.

Protecting your laboratory environment
LABCONCO

Safety Gap Analysis

Chemical Safety Features	Type A2 w/ Canopy	Type B1	Type B2	Type C1
Single-pass airflow	X	✓	✓	✓
Intuitive use of work space	✓	X	✓	✓
Easy installation and building demands	✓	✓	X	✓
Confident chemical safety	X	X	✓	✓
Complete protection during exhaust failure	X	X	X	✓

Table 1. Comparison of the different types of Class II BSC shows the capabilities of each type in different operational status and conditions. The Type C1 provides the most protection and flexibility.

Class II, Type B and C* BSCs are designed for use with both microbiology and chemistry applications. They filter out particles in the air at the cabinet's exhaust outlet, and exhaust hazardous chemical vapors outside of the laboratory.

Know Your Needs

If chemical safety is not a concern for your microbiological processes, then a strictly recirculating Class II Type A2 is more than suitable. However, if chemicals are a concern, a risk assessment should help point you in the direction of which vented Class II BSC is right for you. In today's landscape, it is quite possible that tradeoffs must be made. Be sure to know the laboratory's priorities before making a decision because what is easiest may not be the safest. Alternatively, the safest option may turn out to be beyond the needs of the lab and turn out to heavily tax both your building's capabilities and your budget.

Size, m (ft.)	Cat. No.	Unit
Purifier Logic+ A2 Rediship, 25.4cm (10") Sash with Options and Base Stand		
0.9 (3')	89413-124	Each
1.2 (4')	89413-128	Each
1.5 (5')	89413-132	Each
1.8 (6')	89413-136	Each
Purifier Logic+ B2, 8" Sash with Options and Base Stand		
1.2 (4')	89412-920	Each
1.8 (6')	89412-928	Each
Purifier Axiom C1, 10" Sash with Options and Base Stand		
1.2 (4')	10148-126	Each
1.8 (6')	10148-272	Each

VWR bioMarke™
The market source for Life Science!

VWR Literature Library
C/O Archway
20770 Westwood Drive
Strongsville, OH 44149

ATTN: POST OFFICE: Electronic Service Requested

PRST STD
POSTAGE & FEES
PAID
PERMIT #293
Midland, MI

0416 30M Lit. No. 93595

ATTN: MAILROOM:
If addressee is no longer with the organization, please deliver to laboratory products buyer.
Thank you.

VWR Life Science Cell Culture Media and Reagents

VWR introduces classical media and supporting reagents for cell culture. Researchers looking for a source of classical media for cell culture now have a cost-effective solution available from the VWR Life Science line of products.

Products include

- **DMEM:** Both high glucose and low glucose options are available
- **RPMI:** Available with or without L-glutamine
- **Balanced Salt Solutions and Reagents:** Dulbecco's Phosphate, buffered Saline (DPBS) HBSS, Trypsin, and L-Glutamine

To view additional products for your cell culture workflow, please visit **vwr.com/cell culture.**

NEW