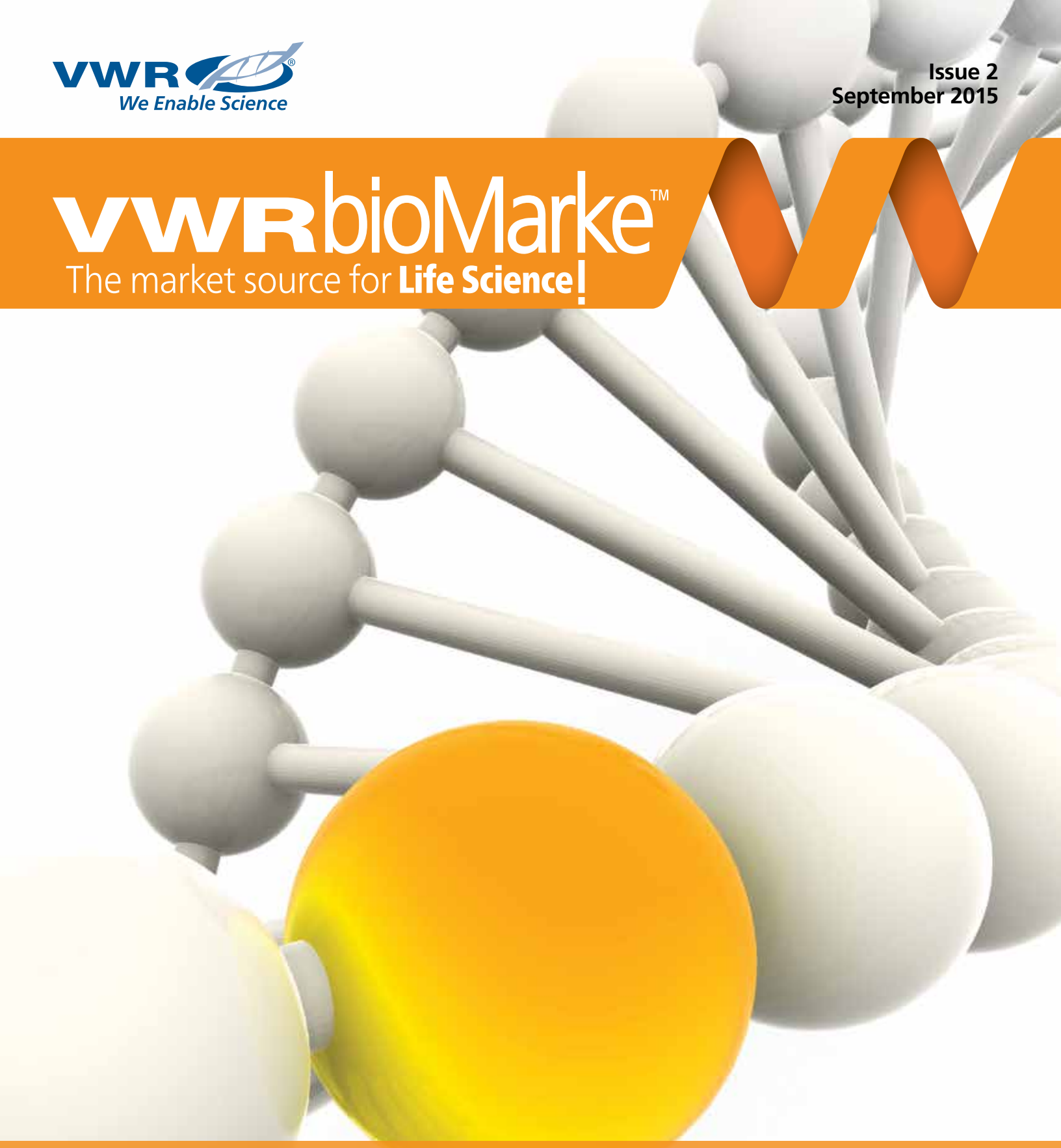
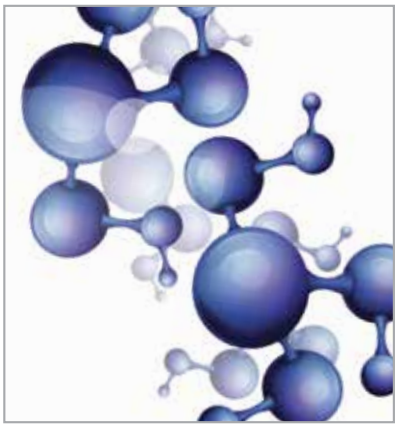
VWR
We Enable Science

Issue 2
September 2015

VWR bioMarket™

The market source for **Life Science!**





CONTENTS

EZ-Vision® Bluelight is a Safe DNA Dye Compatible with Blue-Light Visualization

INTRODUCTION

DNA analysis by agarose gel electrophoresis is a common activity performed in life science labs across the globe. The majority of gels are stained with a dye that intercalates between adjacent base pairs and fluoresces upon exposure to UV light, thus revealing bands of DNA on the gel. Despite its status as a toxic, mutagenic, and potentially carcinogenic substance, the most frequently used dye for DNA visualization since the 1970's has been ethidium bromide. UV exposure, which is known to damage DNA, can negatively impact downstream applications, such as molecular cloning and sequencing. In order to alleviate both the safety concerns and the functional side effects of the ethidium bromide-UV light combination, VWR Life Science AMRESCO developed the newest addition to the EZ-Vision product line, EZ-Vision Bluelight DNA dye.

EZ-Vision Bluelight is a sensitive, non-mutagenic and environmentally safe fluorescent dye that is compatible with gel documentation using blue-light excitation. It is available as a 10,000X concentrated solution that may be used for in-gel or post-gel staining, with sensitivity down to as little as 1–3ng DNA. EZ-Vision Bluelight is the perfect complement to the original EZ-Vision DNA dyes (EZ-Vision One, EZ-Vision Two, and EZ-Vision Three) and the more recent EZ-Vision In-Gel Solution, by offering the same safety benefits, as well as enabling a gel imaging option that does not affect DNA integrity for downstream applications.

METHODS AND RESULTS

Performance of In-gel and Post-electrophoresis Gel Staining Using EZ-Vision® Bluelight

The performance of EZ-Vision Bluelight DNA dye as an in-gel and post-gel stain was assessed by electrophoresis of serially diluted PCR DNA Markers on 1% agarose gels. For in-gel staining, EZ-Vision Bluelight was diluted 1:10,000 into a 1% molten Agarose I™ solution in TAE buffer. The gel was loaded, run to completion, and then immediately imaged with a blue LED transilluminator (410–510nm) and a green filter (500–600nm) (Figure 1A). Post-staining of a 1% Agarose I/TAE gel was performed by incubating the resolved gel for 30 minutes with gentle agitation in EZ-Vision Bluelight diluted 1:10,000 in water. The post-stained gel was then imaged with a blue LED transilluminator (Figure 1B). Staining with EZ-Vision Bluelight produced bright bands on both gels, with nearly identical migration distances of individual bands. While post-gel staining exhibited the sharpest band resolution, the in-gel staining appeared to stain DNA more intensely. EZ-Vision Bluelight also works well as a post-stain for gels (Figure 2).

Figure 1. A) In-gel Staining

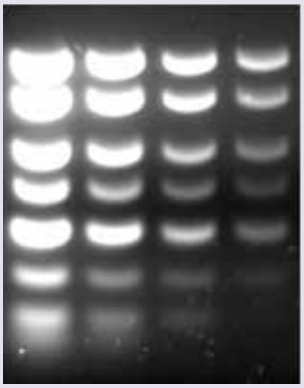


Figure 1. B) Post-gel Staining

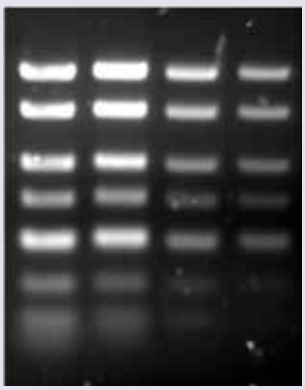


Figure 2.

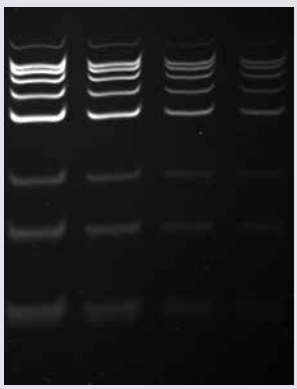


Figure 1. Comparison of in-gel and post-gel staining with EZ-Vision® Bluelight DNA Dye. 1% agarose gels in TAE buffer were prepared **(A)** with 1X EZ-Vision Bluelight DNA dye in-gel or **(B)** without dye in the gel. Both gels were loaded with 2-fold serial dilutions of PCR DNA Marker and electrophoresed in parallel for the same length of time at the same voltage. Immediately after electrophoresis, the **(A)** in-gel stained DNA was imaged with blue-light LED transillumination, while the **(B)** unstained gel was incubated in 1X EZ-Vision Bluelight DNA dye in water for 30 minutes before being imaged by blue-light LED transillumination (410–510nm) and a green emission filter (500–600nm).

Figure 2. Post-staining of a polyacrylamide gel with EZ-Vision Bluelight. A 12% polyacrylamide gel with TBE buffer was run with DNA ranging from 17bp to 102bp. After electrophoresis, the gel was stained in TBE containing 1X EZ-Vision Bluelight for 30 minutes, then imaged using blue-light LED transillumination (410–510nm) and a green emission filter (500–600nm).

Figure 3. Sensitivity of EZ-Vision Bluelight for post-staining of a DNA gel. A 1% agarose/TAE gel with in-gel EZ-Vision Bluelight was run with 2-fold dilutions of DNA. A 500bp fragment was detected from 47ng to 2.94ng by blue-light LED transillumination (410–510nm) and a green emission filter (500–600nm).

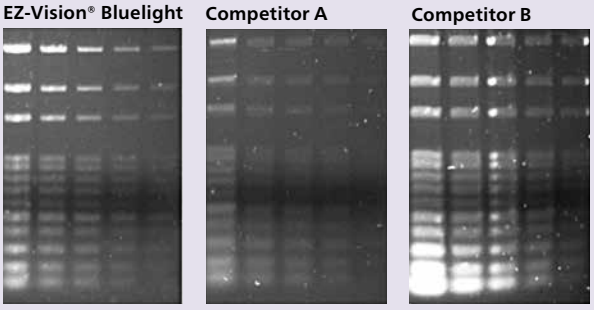


Figure 4. Comparison of EZ-Vision® Bluelight with leading competitors' DNA dyes for blue-light visualization. Three 1% agarose gels in TAE buffer were loaded with identical samples and electrophoresed under identical conditions. The gels were then post-stained according to the manufacturer's instructions with **(A)** EZ-Vision® Bluelight DNA Dye, **(B)** Competitor A and **(C)** Competitor B. The gels were imaged with blue-light LED transillumination (410–510nm) and a green emission filter (500–600nm).

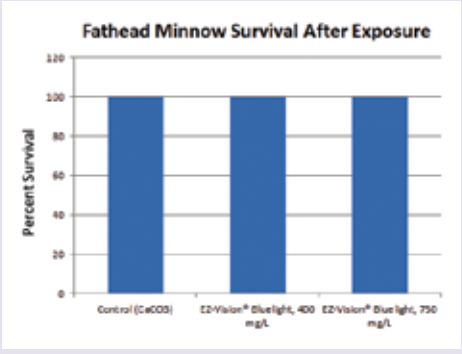


Figure 5. Environmental hazard testing of EZ-Vision Bluelight DNA Dye. EZ-Vision Bluelight environmental hazard testing was performed using the CCR Title 22 Fathead Minnow Hazardous Waste Screen Bioassay. The percent survival after 96 hours exposure to EZ-Vision Bluelight or the control substance, $CaCO_3$, is shown. EZ-Vision Bluelight was determined to be non-hazardous in this assay, with a LC_{50} > 750mg/L. All fish in the assay survived after 96 hours of exposure to EZ-Vision Bluelight.

Staining is effective in standard TAE and TBE running buffers, as well as water. Destaining is not required.

EZ-Vision Bluelight DNA Dye may be used for sensitive and UV-free detection of DNA Sensitivity of EZ-Vision Bluelight staining for detection of DNA was analyzed using a 500bp DNA fragment that was resolved on a 1% agarose gel precast with 1X dye. The 500bp fragment was visualized immediately after electrophoresis on a blue LED transilluminator with a green filter. A band was observed for 500bp DNA ranging from 47ng down to 2.94ng (Figure 3). This sensitivity is similar to that of ethidium bromide, but without the toxicity of the dye itself or the need for exposure to ultra violet light. By using blue light excitation during gel imaging, DNA integrity for downstream applications is maintained because, unlike UV light, it does not induce nicking of samples.

Comparison of EZ-Vision Bluelight with Leading Competitors' DNA Dyes for Blue-light Visualization

A direct comparison of EZ-Vision Bluelight with two competitive bluelight dyes was performed by running identical samples of DNA MW Marker, 100bp on unstained 1% agarose gels in TAE buffer. Following electrophoresis, the gels were stained according to their respective DNA dye instructions. All gels were then imaged with blue-light transillumination with a green filter. The EZ-Vision Bluelight stained gel appeared most comparable to the gel stained with Competitor A (Figure 4).

EZ-Vision Bluelight is a Safe, Non-mutagenic, Non-toxic Alternative to Ethidium Bromide

EZ-Vision Bluelight DNA Dye was tested by an independent entity, Aquatic Testing Laboratories (Ventura, CA), for environmental safety according to the CCR Title 22 Fathead Minnow Hazardous Waste Screen Bioassay. This assay gauges the potential hazard of releasing a substance in the environment by monitoring its toxicity in aquatic organisms, specifically fathead minnows. The results were measured as the percent survival of the fish at specified concentrations of EZ-Vision Bluelight DNA dye, or a control substance ($CaCO_3$) after 96 hours of exposure. For designation as a non-hazardous substance, the LC_{50} must be greater than 500mg/L. EZ-Vision Bluelight DNA Dye was determined to be non-hazardous since 100% of fish survived after 96 hours of exposure and

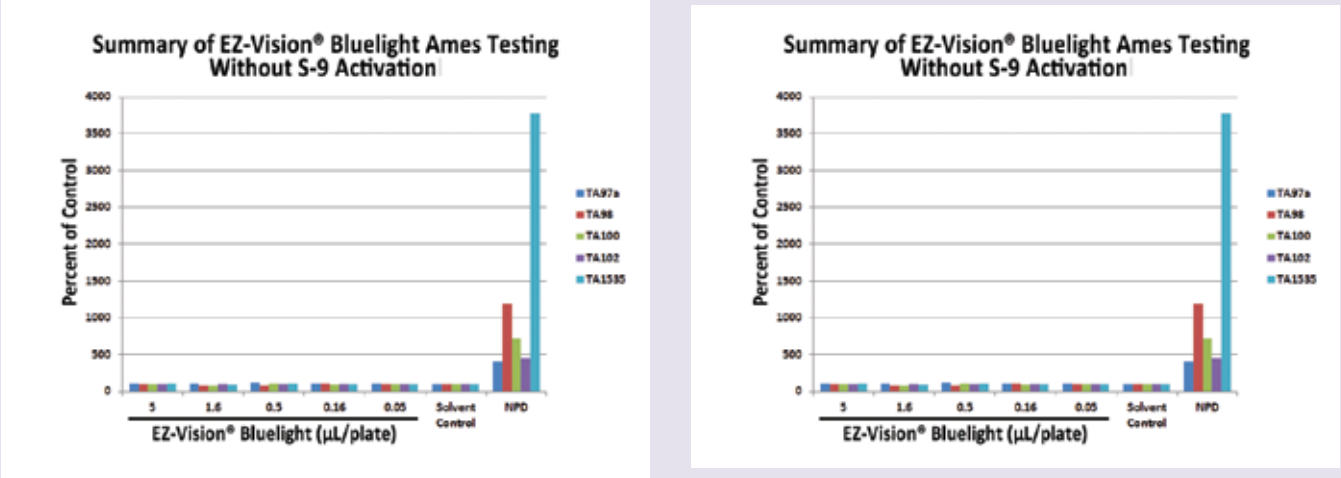


Figure 6. Mutagenicity testing of EZ-Vision Bluelight DNA Dye. EZ-Vision Bluelight does not meet the criteria for classification as a potential mutagen as determined by the Ames test. Exposure to EZ-Vision Bluelight did not significantly increase the percentage of revertants of mutant *S. typhimurium* over controls either (A) without or (B) with S-9 activation. NPD = mutagenic control, 4-nitro-0-phenylene-diamine.

the LC_{50} was found to be greater than 750mg/L (Figure 5).

Mutagenicity testing of EZ-Vision Bluelight was performed by Nelson Laboratories (Salt Lake City, UT) using the Ames test, which monitors the ability of various histidine-dependent mutant strains of *Salmonella typhimurium*, to revert to histidine independent bacteria. This reverse mutation assay was performed both with (Figure 6A) and without (Figure 6B) metabolic activation using an S-9 activation system, which mimics mammalian metabolic conditions. The S-9 activation system helps identify substances that are metabolized from a non-mutagenic state to a mutagenic by-product. The criteria for classification as a non-mutagenic substance included the reverse mutation rates being less than or equal to a defined percentage of the solvent control used for each of the mutant *S. typhimurium* strains and the absence of dosage responsiveness for different dilutions of the tested substance. EZ-Vision Bluelight meets the criteria of a non-mutagenic substance because no significant increase in reverse mutations was found in bacteria treated with EZ-Vision Bluelight, nor was there a dose response to the five dilutions of EZ-Vision Bluelight used (Figure 6).

CONCLUSIONS

Researchers worldwide are already benefitting from the switch to EZ-Vision products for DNA, which includes EZ-Vision One, Two, and Three DNA dyes, EZ-Vision In-Gel Solution, and the newest addition, EZ-Vision Bluelight DNA dye. All of these contain a safe, non-mutagenic dye that enables gels to be handled without exposing lab personnel or the environment to hazards. EZ-Vision Bluelight offers further safety through its compatibility with blue-light imaging to eliminate the need to use ultra-violet transillumination, which can harm the operator as well as damage the DNA being analyzed. The sensitivity of all EZ-Vision DNA dyes is similar to that of ethidium bromide and they are fully compatible with downstream applications, making them an ideal choice for DNA visualization.

Description	Size	Cat. No.
EZ-Vision Blue Light DNA Dye	500 µL	**10791-798**

Safe Gel Imaging Using GelGreen® Dye and UVP BioImaging Systems

Gel imaging and nucleic acid binding dyes are widely used in today's life science laboratories to visualize DNA fragments in agarose gels. Ethidium bromide (EtBr) has been the predominant dye used for nucleic acid gel staining for decades because of its low initial price and generally sufficient sensitivity. However, the safety hazards and costs associated with decontamination and waste disposal can ultimately make the dye comparatively expensive and unsafe to use. For this reason, GelGreen™, a next-generation fluorescent nucleic acid gel stain, was developed by scientists at Biotium, Inc. Three attributes make GelGreen dye superior to EtBr and other EtBr alternatives: low toxicity, high sensitivity, and exceptional stability. GelGreen offers the added advantage of imaging using visible blue light instead of potentially harmful UV illumination.

GelGreen, a nucleic acid binding dye, can be precast in agarose gels or used to stain gels after electrophoresis. Once nucleic acid samples are separated by electrophoresis and stained, UVP's GelDoc-It®TS2 and ChemiDoc-It®TS2 Imaging Systems can be used to image the fluorescent bands using the UVP 3UV™ Transilluminator with 302/365/254nm UV excitation, Visi-Blue blue light plate and a green fluorescence emission filter (Fig. 1).

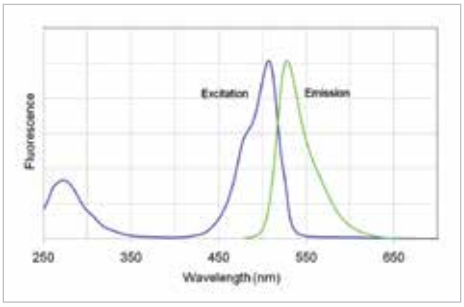


Figure 1. Excitation and emission spectra of GelGreen dye bound to dsDNA.

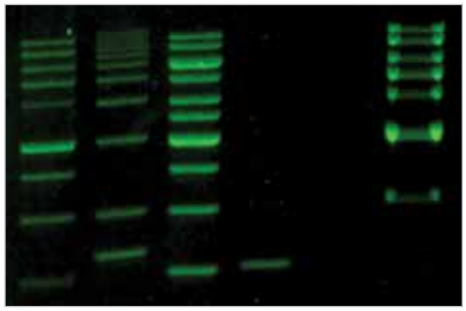


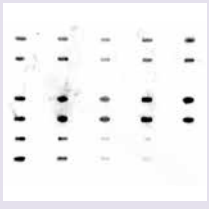
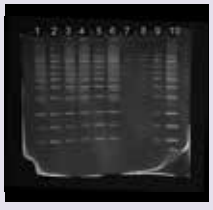
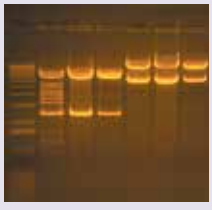
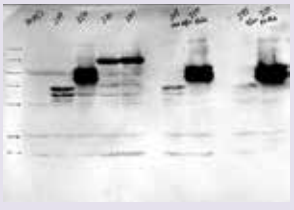
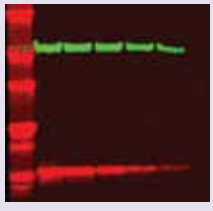
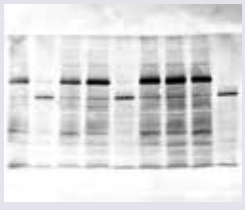
Figure 2. GelGreen post-stained gel imaged using the Visi-Blue converter plate and UVP GelDoc-It^{TS2}.

The Visi-Blue plate converts 302nm UV light to 460–470nm blue light, protecting researchers from exposure to harmful UV irradiation during band excision and preventing UV damage to DNA, which may dramatically reduce subsequent cloning efficiency. As shown in the resultant image (Fig. 2), exceptionally clear and quantifiable bands are detected using the GelDoc-It^{TS2} and ChemiDoc-It^{TS2} Imaging Systems on gels stained with GelGreen dye.

System	Camera/Lens	Transilluminator	Cat. No.
GelDoc-It^{TS2} 310	2.0 MPX Non-Cooled f/1.2 12.5-75 mm zoom	302 nm UV	**71004-576**
GelDoc-It^{TS2} 310	2.0 MPX/Non-Cooled f1.2 12.5-75 mm zoom	254/302/365 nm UV	**71004-578**
ChemiDoc-It^{TS2} 810	8.1 MPX/Cooled 30 mm lens	302 nm UV	**71003-042**
ChemiDoc-It^{TS2} 810	8.1 MPX/Cooled 30 mm lens	254/302/365 nm UV	**71004-608**
ChemiDoc-It^{TS2} 810	8.1 MPX/Cooled 50 mm lens	302 nm UV	**71004-604**
ChemiDoc-It^{TS2} 810	8.1 MPX/Cooled 50 mm lens	254/302/365 nm UV	**71004-606**
ChemiDoc-It^{TS2} 510	2.1 MPX/Cooled f/1.2 12.5-7 5mm zoom	302 nm UV	**89422-800**
ChemiDoc-It^{TS2} 510	8.1 MPX/Cooled f/1.2 12.5-75 mm zoom	254/302/365 nm UV	**89422-798**

Easy to Use Gel and Blot Imaging at Your Fingertips!

Fluorescence, chemiluminescence, colorimetric and multiplex applications utilizing ultraviolet, visible, and near-IR spectrums.

Advanced Touch Screen Gel/Blot Imaging

UVP
An Analytik Jena Company

Cooled and Non-Cooled CCD Cameras (upgradeable), built into the cabinet, offer high sensitivity for applications including chemiluminescence blot and gel imaging.

Fast fixed or zoom lens options provide the superior sensitivity to film.

Large Touch Screen is integrated into the system with a generous 15.6" for maximizing imaging functionality.

TS2 Software with intuitive control panel simplifies the imaging workflow; users can set language of choice and design templates for repeat, time saving experiments.

Emission Filters are placed in the easy access five-position filter tray. An ethidium bromide filter is standard; additional filters are available.

Side Port provides access for the optional BioLite MultiSpectral Light Source (not shown). BioLite supplies powerful epi illumination with selectable wavelengths from visible to NIR spectrum.

USB and SD Ports are located on the side of the darkroom for saving images; or connect to a wired or wireless network to transfer images to another computer.

Light-Tight Cabinet is ideal for chemiluminescent imaging applications.

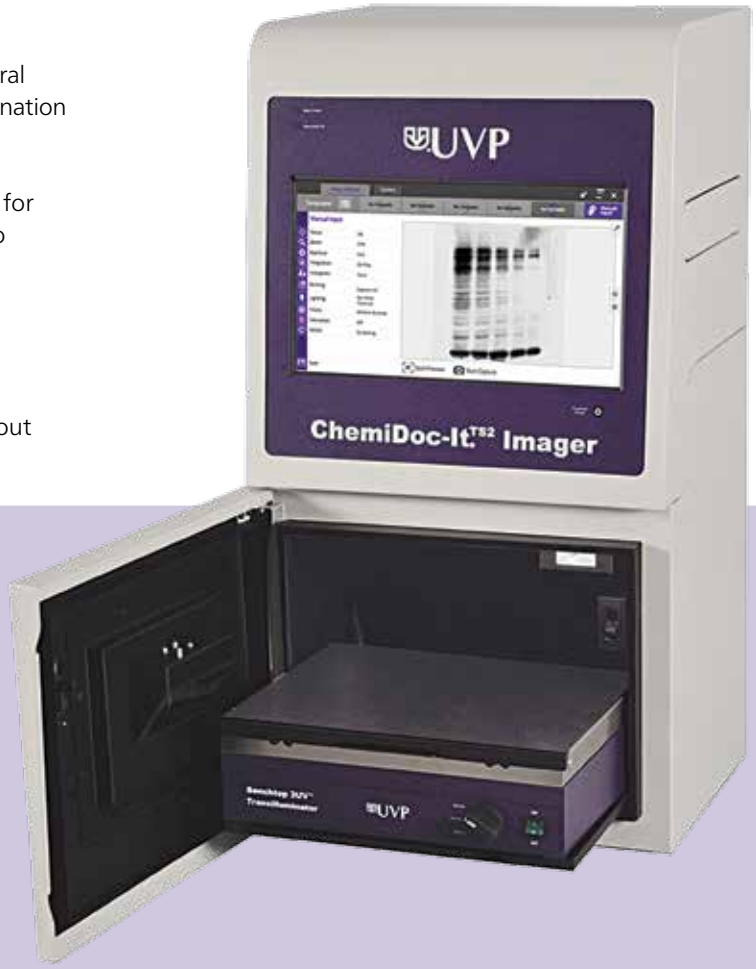
Viewing Window (UV blocking) for viewing samples without opening the cabinet door.

Chemi Tray, included with the ChemiDoc-It^{TS2}, enables placement of samples such as chemiluminescent Western blots.

Transilluminator is placed on the easy access roll-out tray; choose from models with single UV or multiple UV wavelengths and filter sizes from 21x26cm to 25x26cm.

VisionWorksLS Quantitative Analysis Software, with unlimited user-license copies, is provided for loading on additional PCs. Software enables 1D analysis of lanes and bands, area density analysis, annotation, and reports.

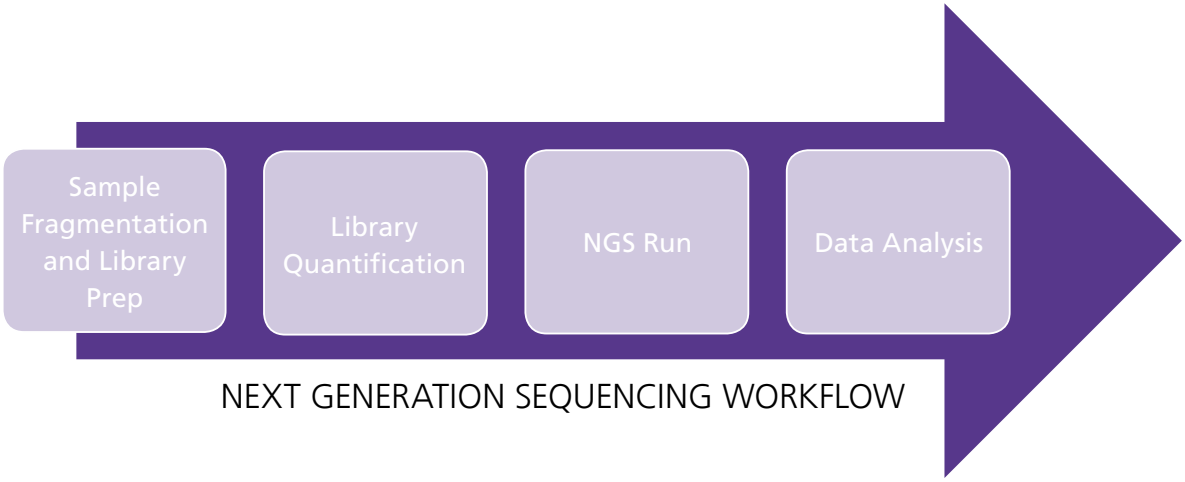
Visi-Blue™ Light Plate can be added to the systems to supply 460–470nm blue light for excitation of stains such as GelGreen™, SYBR™ Green, and SYBR Safe.



PerfeCTa® NGS Quantification Technology:

Accurate Library Quantification for Next Gen Sequencing

- Accurate and sensitive method for NGS library quantification
- Stabilized, prediluted standards for convenient use
- Consistency across a broad range of samples
- Sensitivity of detection to quantify low concentration libraries
- Quantification for either Illumina or Ion Torrent



Accurate quantification of the number of amplifiable library molecules is the most critical step in the NGS workflow in obtaining high quality read data with next-generation sequencing technologies. The PerfeCTa NGS Library Quantification Kit uses real-time PCR to specifically quantify library molecules that possess the appropriate adapter tag at each end. These are the suitable template molecules for either emulsion PCR used for the Ion Torrent platform or Bridge PCR used for Illumina NGS platforms.

PerfeCTa NGS Quantification kits simplify the library quantification process by providing stabilized, pre-diluted standards, pre-qualified primer sets, and an optimized dilution buffer for your NGS library samples. This minimizes pipetting errors and ensures reproducible and precise qPCR results, even with dilute samples. The robust qPCR performance of PerfeCTa SYBR Green SuperMix provides accurate quantification of NGS libraries with varying fragment sizes or GC content. Kits are available to support all major qPCR instrument platforms.

NGS Library Quantification Kit components:

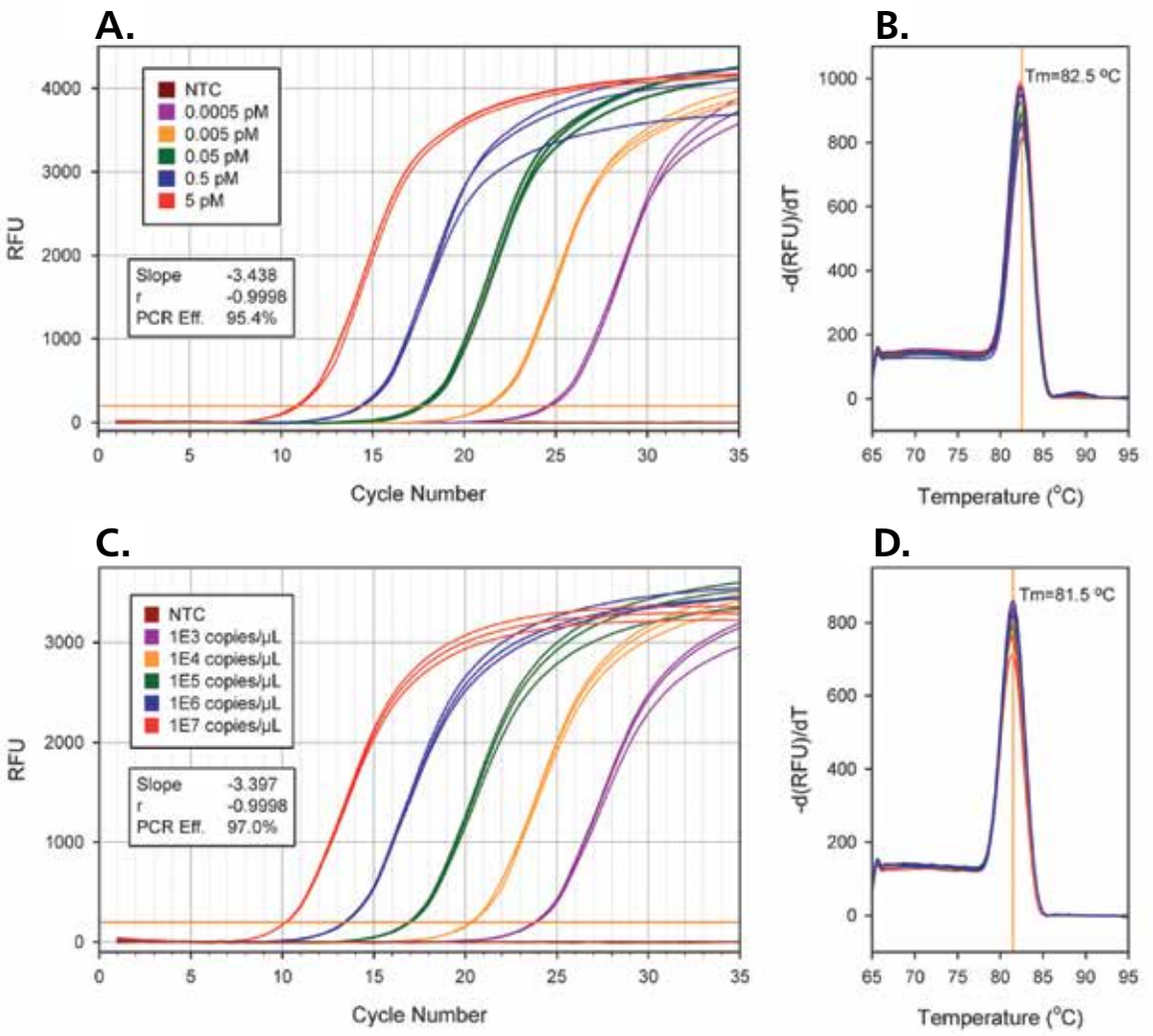
- NGS Primer Mix (10µM)
- Pre-diluted standard DNAs
- Dilution Buffer for NGS library samples
- Bundled with one of the following:

PerfeCTa SYBR Green SuperMix
PerfeCTa SYBR Green SuperMix with ROX
PerfeCTa SYBR Green SuperMix Low ROX

A common problem with some NGS library quantification protocols is the use of DNA standards that are too concentrated and, as a result, generate qPCR data that fall outside the linear dynamic range for many qPCR instruments. Improper baseline settings result in compressions between the highest concentrated DNA standards that give rise to inflated PCR efficiencies and inaccurate library quantification results. The NGS DNA standards supplied with the PerfeCTa NGS Library quantification kits have been carefully selected to avoid these artifacts and produce NGS library standard curves with exceptionally high linear regression correlation coefficients.

The DNA standard for Illumina NGS platforms generates a 426bp amplicon (48.8% GC). Primer sequences correspond to the "P5" and "P7" primer sequences for Illumina sequencing libraries:

Quanta BIOSCIENCES™



Illumina forward primer:
5' AAT GAT ACG GCG ACC ACC GA 3'
Illumina reverse primer:
5'-CAA GCA GAA GAC GGC ATA CGA-3'

The Ion Torrent DNA standard produces a 183bp amplicon (51.9% GC) using primers that target the "A" and "trP1" adaptor sequences:

Ion Torrent forward:
5'-CCA TCT CAT CCC TGC GTG TC-3'
Ion Torrent reverse:
5'-CCT CTC TAT GGG CAG TCG GTG AT-3'

PerfeCTa NGS library Quantification Kit performance data. qPCR amplification of each of the five supplied DNA standards for Illumina NGS libraries (panel A) or Ion Torrent libraries (panel C) were carried out with the supplied primer sets (300nM final concentration) and PerfeCTa SYBR® Green SuperMix in 20µL reaction volumes on a Bio-Rad CFX-96. Reactions were incubated for 5 min at 95˚C followed by 35 cycles of: 95˚C, 10s; 60˚C, 20s; 72˚C, 45s. Real-time fluorescence data was collected and analyzed at completion of the 72˚C extension step. After completion of PCR, a dissociation (melt) curve was performed to verify amplification of a single specific product (panels B and D).

PerfeCTa NGS Quantification Kit	Size	Cat. No.
PerfeCTa NGS Quantification Kit - Ion Torrent	500 x 20 µL Reactions	**10029-552**
PerfeCTa NGS Quantification Kit - Ion Torrent/ROX	500 x 20 µL Reactions	**10029-554**
PerfeCTa NGS Quantification Kit - Ion Torrent/Low ROX	500 x 20 µL Reactions	**10029-556**
PerfeCTa NGS Quantification Kit - Illumina	500 x 20 µL Reactions	**10029-558**
PerfeCTa NGS Quantification Kit - Illumina/ROX	500 x 20 µL Reactions	**10029-546**
PerfeCTa NGS Quantification Kit - Illumina/Low ROX	500 x 20 µL Reactions	**10029-550**

Automated Extraction of Circulating DNA from Plasma/Serum

OMEGA bio-tek

Simple blood draws are replacing risky procedures such as amniocentesis and tumor biopsies thanks to the discovery of circulating cell-free DNA (cfDNA). Typically less than 300 base pairs in length, cfDNA from the developing fetus or from tumor growth accumulates in the blood stream.

Circulating cfDNA provides a rich non-invasive source of genetic information to help evaluate the health of the fetus or the progression of cancer. Another advantage of cfDNA is that it can be readily obtained from plasma or serum with a targeted and tailored extraction process. This avoids the two major drawbacks of using whole blood as a diagnostic specimen: the inhibitory substances from red blood cells and the overabundance of maternal or host DNA from white blood cells. These can carry over during the DNA extraction process and interfere with downstream diagnostic testing.

As the demand for circulating DNA increases, the need for automation to increase throughput and decrease user error is paramount. The Mag-Bind Circulating DNA Kit can be used in combination with 24-well microtiter plates and liquid handling instruments, with individual probes such as the Hamilton MicroLab STAR or Tecan Freedom Evo instruments for fully automated extractions. Magnetic stands can be placed on the instruments SBS format positions. Magnetic processors such as the Thermo Kingfisher Flex with 24-well magnet can also be utilized for semi-automated extractions.

Figure 1 below shows the Mag-Bind Circulating DNA kit has better recovery than other commercially available silica column based products on the market. Host DNA contamination can cause issues with downstream analysis and results. Figure 2 shows that the Mag-Bind Circulating DNA Kit preferentially recovers smaller DNA fragments thus minizing host DNA co-purification.

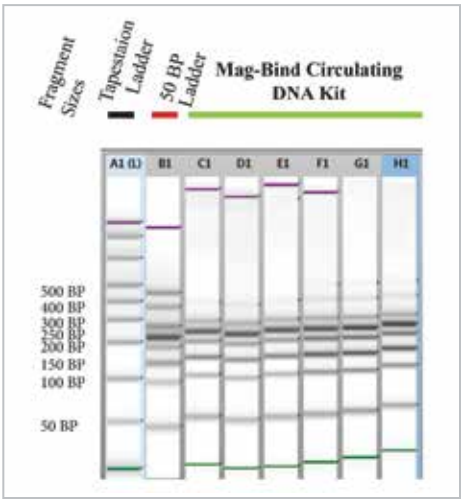
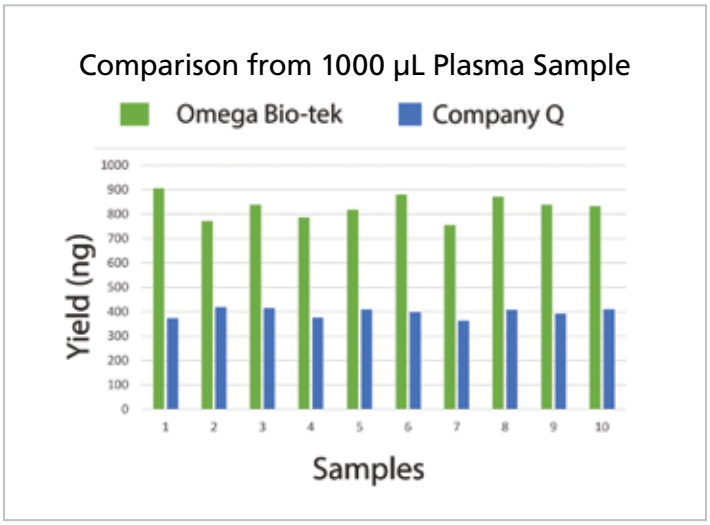


Figure 2. 10µL 50bp ladder was spiked into 1mL plasma and isolated using the Mag Bind Circulating DNA Kit. Preferential binding was observed for fragments smaller than 300bp.

Contact your VWR Rep for more information on these products.



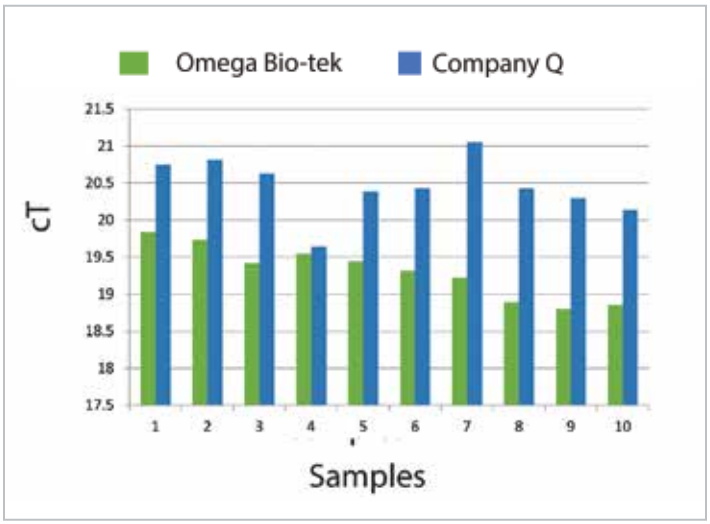


Figure 1. DNA yield from 1000µL plasma extracted using the Mag Bind Circulating DNA Kit and Company Q using the manufacturer's recommended protocols. Quantification done via Picogreen. ~200 BP fragment amplified with Quanta Biosciences PerfeCTa® SYBR® qPCR reagents targeting 18s ribosomal DNA.

Decontamination of the KingFisher Duo Prime Processing Chamber Using UV Light

SP&A Application Laboratory, Thermo Fisher Scientific, Vantaa, Finland

ABSTRACT

The new Thermo Scientific™ KingFisher™ Duo Prime magnetic particle processor contains an ultraviolet C (UVC) light inside the instrument to eliminate most bacterial and viral contaminants caused by sample handling. The UVC irradiation induces damage to the DNA of the organisms, thereby inhibiting their growth. The light is designed to reach over the entire work surface of the instrument to quickly eliminate contaminants. This note represents how easily bacterial growth is inhibited by the UVC irradiation inside KingFisher Duo Prime. The deactivation of genomic DNA subjected to UVC irradiation prior to a PCR amplification is also shown.

INTRODUCTION

Short wavelength ultraviolet light between 200–280nm, classified as UVC light, is generally accepted to be germicidal and inactivates bacterial growth effectively. The optimal germicidal wavelengths are in the range of 255–265nm. The KingFisher Duo Prime instrument contains a mercury lamp with peak emission around 255nm. Micro-organisms are destroyed due to the UVC radiation causing DNA lesions, which block transcription and replication with lethal consequences. An example of the UVC exposure effect on DNA is shown in Figure 1. The efficiency of nucleic acid inactivation depends on the organism. The specific genetic composition and other molecules may protect the organism from UVC-induced damage. Bacteria and viruses are readily inactivated, whereas fungal cells and spores from both bacteria and fungi typically require higher UV dosages[2].

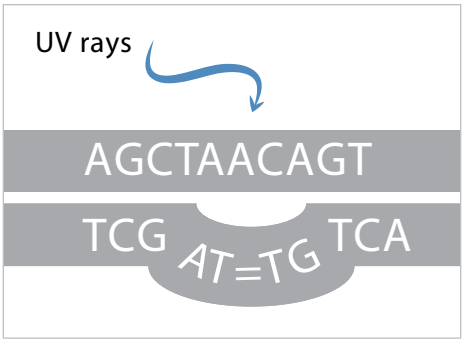


Figure 1. An example of DNA lesion caused by UVC radiation. Thymine bases cross-link, preventing DNA replication. The cross-links may also occur between adjacent strands of nucleic acids or protein molecules.

In this application note, the lethal effect of UVC irradiation on bacterial growth is shown, as well as the deactivation of human genomic DNA (gDNA). A typical laboratory bacteria, *E. coli,* was used to verify the inhibition efficiency of the UV lamp inside the KingFisher Duo Prime. The gDNA deactivation efficiency of KingFisher Duo Prime UV lamp was compared to a commonly used PCR UV Cabinet.

MATERIALS AND METHODS

Power of the UVC Lamp

Inside the KingFisher Duo Prime instrument, there is an 8.0W UV bulb. The output power of the lamp in the UVC spectra is 2.0W. In comparison to other similar-type automated DNA purification instruments, the UVC power of the KingFisher Duo Prime instrument is significantly higher (Table 1).

	UV bulb electrical power	UVC radiation power
KingFisher Duo Prime	8.0 W	2.0 W
Competitor M*	4.5 W	0.8 W

Table 1. Technical specifications of the UV lamp integrated in the DNA purification instrument
* Information based on manufacturer specifications accessed April 2015.

Microbial Growth Inhibition

An *E. coli* BL-21 bacterial strain was grown in Tryptone Soya Broth for four hours to exponential phase. The grown bacteria was diluted 10^{-5} and 100µL was plated onto Tryptone Soya Agar Petri dishes. Five bacteria containing agar dishes were placed to cover the working surface of three KingFisher Duo Prime instruments (Figure 2).

A control dish containing the same amount of bacteria was placed into equal environmental conditions outside the instrument. The bacteria were subjected to 30 minutes of UVC exposure inside the instrument. The control dish and the UVC-exposed dishes were incubated overnight at 37°C. The next day the colonies were counted with OpenCFU software[1]. (Figure 3).

Genomic DNA Deactivation

Human genomic DNA was subjected to UV light to determine the amount of inhibition UV would cause to the PCR amplification of the template. The UVC/T-M-AR PCR UV Cabinet (from Grant Instruments, Cambridge, UK) was used as a reference for the effect of UVC irradiation.

24-well PCR plates with dried human gDNA were placed inside three KingFisher Duo Prime instruments and the UV lamp was set for 16 hours. Similar settings were done with the PCR UV Cabinet. Positive controls on the PCR plates were covered during the UV irradiation to protect the samples from UV rays. Two samples and one positive control with 10,000 copies of human gDNA, all in three replicates, were used for each instrument.

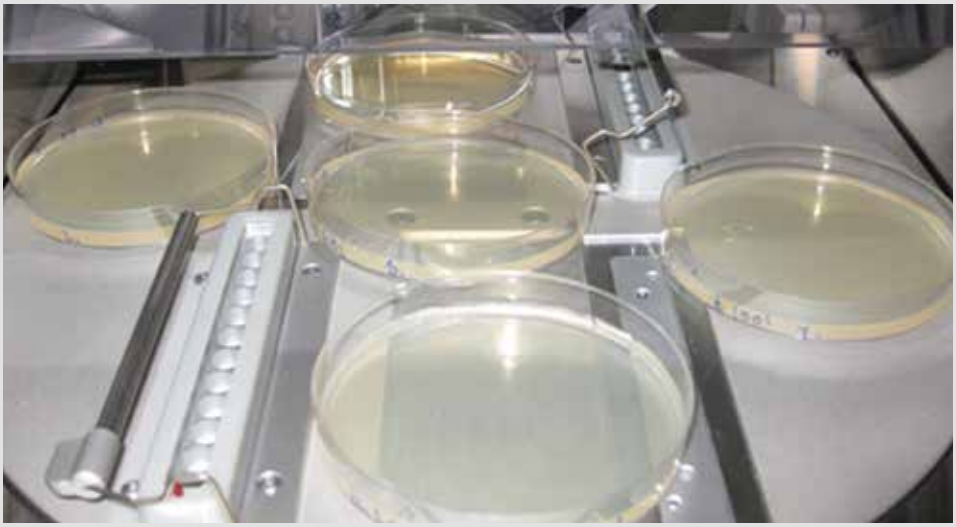


Figure 2. The orientation of the bacteria-containing agar dishes on the KingFisher Duo Prime round table.

	Standards				UV-irradiated Samples		Pos. Control
NTC	10	100	1,000	10,000	10,000	10,000	10,000
NTC	10	100	1,000	10,000	10,000	10,000	10,000
NTC	10	100	1,000	10,000	10,000	10,000	10,000

Table 2. The orientation of samples on the 24-well Piko PCR Plate.

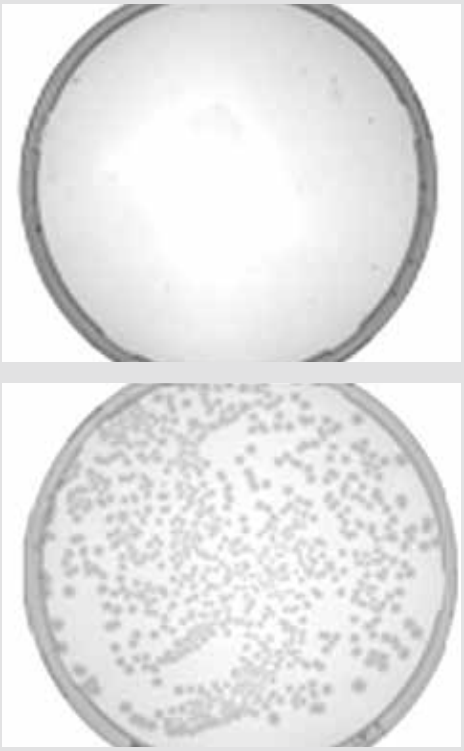


Figure 3. The UV-irradiated agar dish, top, and the control dish, bottom.

As standards, the 1:10 serial dilution of the gDNA was prepared and NTC was added into each PCR plate, all in three replicates (Table 2). Cathepsin K gene amplification was determined using SYBR® qPCR reagents with a real-time PCR system.

RESULTS

Microbial Growth Inhibition Test

The colony count on the control dish outside the instrument had roughly 700 colonies. The number of colonies on the UVC exposed dishes is shown in Table 3. The average number of bacterial colonies on the test plate was 0.27: (1+1+2)/15 = 0.27. In comparison, the number of colonies on the control plate was 700. Therefore the reduction in colony count due to the UVC exposure was 99.96%: 100%-(0.27/700x100) = 99.96%.

KingFisher Duo Prime with UVC light is highly efficient for decontaminating the instrument from bacteria and genomic DNA.

Genomic DNA Inactivation

The effect of UVC irradiation to gDNA was determined by calculating the difference of the qPCR amplification in the UVC-treated sample to the amplification of the positive control. PikoReal software was used to analyze the amplification. The example of the amplification from one qPCR run is shown in Figure 4. The average Cq values of the UV irradiated samples and positive controls from three KingFisher Duo Prime

Thermo SCIENTIFIC

KF Duo Prime	Dish Replicate No.				
	1	2	3	4	5
A	1	0	1	0	0
B	0	0	0	0	0
C	0	0	0	0	2

Table 3. The number of bacterial colonies on the agar dishes after 30 minutes of UVC exposure in three parallel KingFisher Duo Prime instruments (A, B, and C).

Instruments	Sample	Cq Average	ΔCq	Average ΔCq
KF Duo Prime A	gDNA UV_1	33.17	11.13	10.7
	gDNA UV_2	32.34	10.30	
	gDNA +ctrl	22.04		
KF Duo Prime B	gDNA UV_1	34.19	11.99	12.2
	gDNA UV_2	34.70	12.49	
	gDNA +ctrl	22.20		
KF Duo Prime C	gDNA UV_1	32.97	10.85	11.2
	gDNA UV_2	33.75	11.63	
	gDNA +ctrl	22.12		
UV Cabinet	gDNA UV_1	33.68	11.67	12.4
	gDNA UV_2	35.14	13.14	
	gDNA +ctrl	22.00		

Table 4. The Cq values of the UVC irradiated gDNA samples and the positive controls treated in three KingFisher Duo Prime instruments and the PCR UV Cabinet.

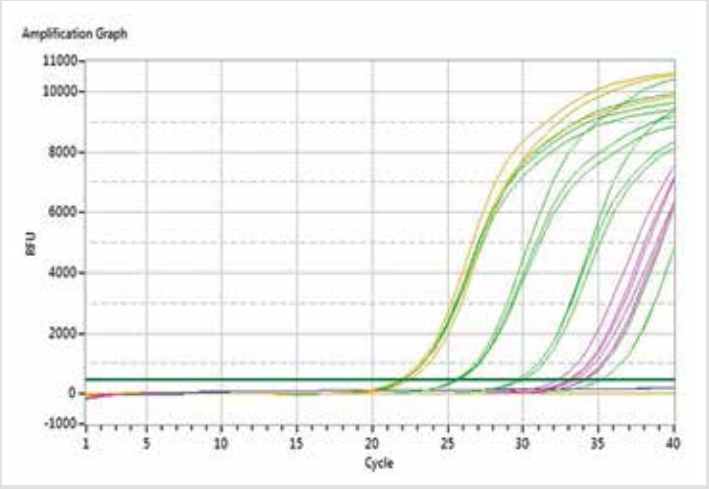


Figure 4. The amplification graph from PikoReal Software (samples from KF Duo Prime C). Colors of the curves: purple – UVC treated samples; orange – positive controls; green – 1:10 serial dilution standards; and blue – NTC.

instruments and the PCR UV Cabinet are listed in Table 4. Average values are from three replicates.

The average Cq value difference (ΔCq) between UV irradiated samples and the gDNA control sample from three instruments was 11.4. The effect of the UVC irradiation can be calculated as follows: 2(- ΔCq) = 0.0004 = 0.04%. The 99.96% reduction in the amplifiable genomic target with this assay was achieved. In comparison, the amplification reduction was 99.98% in the UV PCR Cabinet.

CONCLUSIONS

KingFisher Duo Prime with UVC light is highly efficient for decontaminating the instrument from bacteria and genomic DNA. The UVC irradiance level is on par with that of a typical PCR UV Cabinet and works well in practice.

References

1. *Geissmann Q. OpenCFU, a New Free and Open-Source Software to Count Cell Colonies and Other Circular Objects. PLoS ONE 8(2).*
2. *Kowalski W. Ultraviolet Germicidal Irradiation Handbook: UVGI for Air and Surface Disinfection. Springer, English, 2009.*

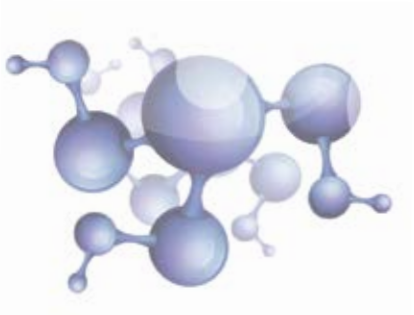
Description	Cat. No.
KingFisher Duo Prime System	10755-330
Barcode Reader	10755-332
Elution Strips, Pack of 40	10755-334

Improved Methods for Site-Directed Mutagenesis Using NEBuilder® HiFi DNA Assembly Master Mix

INTRODUCTION

Site-directed mutagenesis (SDM) is a commonly-used technique for introducing mutations into a gene of interest. When multiple site-directed mutagenesis is needed, existing techniques, such as whole plasmid SDM, are a time consuming option that can be prone to off-target mutation incorporation. This can be a serious impediment to the planning and execution of SDM experiments.

To overcome this issue, NEB® has developed a protocol using NEBuilder HiFi DNA Assembly Master Mix to simplify the construction of single/multiple site-directed mutagenesis. The technique, which involves the design of complementary flanking primers to align fragments, can be readily adapted for SDM applications. In addition, it is unnecessary to use phosphorylated primers for assembly, reducing both cost and time. In one step, two or more PCR products with overlapping ends can be assembled into a vector. An exonuclease creates single-stranded 3′ overhangs that promote annealing of complementary fragments at the overlap region. A polymerase then fills in the gaps, which are then sealed by the DNA ligase. By introducing multiple complementary mutations in the primers at the overlap region, the NEBuilder HiFi DNA Assembly Master Mix forms a single, covalently bonded DNA molecule, containing the desired mutations, which can then be directly transformed into competent cells and screened or sequenced.



Here we describe the use of the NEBuilder HiFi DNA Assembly Master Mix to generate multiple site-directed mutagenesis at the same time.

PROTOCOL

Experimental Design

In this experiment, multiple primers were designed to incorporate three mutations within the gene (Table 1). The resulting amplicons contained 18–20bp overlaps and the desired mutations (Figure 1). The PCR products and linearized vector are treated with the NEBuilder HiFi DNA Assembly Master Mix, and the resulting transformants are screened for mutations by restriction enzyme digestion.

Materials

- NEBuilder HiFi DNA Assembly Master Mix
- Q5® Hot Start High-Fidelity 2X Master Mix
- EcoRI-HF®

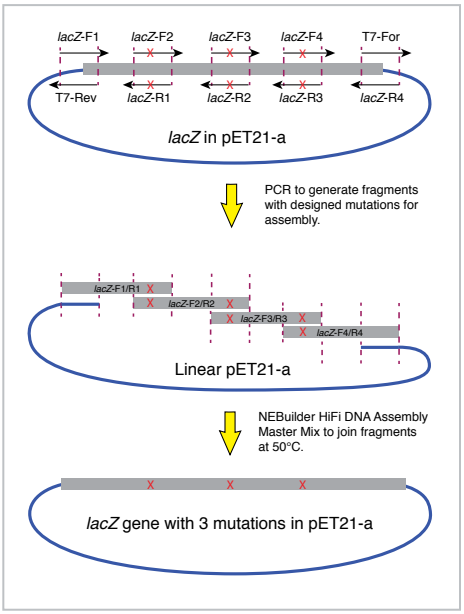


Figure 1. Site-directed mutagenesis of *lacZ* using NEBuilder HiFi DNA Assembly. In this example, multiple mutations are introduced into the *lacZ* gene through overlapping primers followed by PCR. NEBuilder HiFi DNA Assembly is then used to join the fragment with linearized vector.

- CutSmart® buffer (10X)
- Deoxynucleotide Solution Mix
- Overlapping primers
- *lacZ* in pET21a
- DpnI
- NEB 5-alpha Competent *E. coli*
- LB-Amp plates

Table 1. Overlapping primers used for SDM of *lacZ**

PRIMER	Sequence 5′→ 3′
lacZ-F1	TTTAAGAAGGAGATATACATATGACCATGATTACGGATTC*
lacZ-R1	CACATCTGGAATTCAGCCTCCAGTACAGC**
lacZ-F2	AGGCTGAATTCCAGATGTGCGGCGAGTT
lacZ-R2	GGCCTGATGAATTCCCCAGCGACCAGAT
lacZ-F3	CTGGGGAATTCATCGCCCACGGCGC
lacZ-R3	ACACTGAGGAATTCCGCCAGACGCCA
lacZ-F4	TGGCGGAATTCCTCAGTGTGACGCTCCC
lacZ-R4	TTTGTTAGCAGCCGGATCTCATTTTTGACACCAGACCAACT
T7-For	TGAGATCCGGCTGCTAACAAAG
T7-Rev	ATGTATATCTCCTTCTTAAAGTTAAACAAAAT

Red indicates changed nucleotides.

NEW ENGLAND BioLabs® Inc.

Table 2. Fragment preparation specifications.

Component	Volume (µl)
Forward primer (10 µM)	2.5
Reverse primer (10 µM)	2.5
lacZ in pET21a (5 ng/µL)	1.0
ddH_2O	19.0
Q5 Hot Start High-Fidelity 2X Master Mix	25.0
Total Volume	50.0

Step	Temp	Time
Initial denaturation	98°C	1 minute
30 Cycles	98°C	10 seconds
	55°C	15 seconds
	72°C	40 sec. or 3 min
Final extension	72°C	5 minutes
Hold	4°C	∞

Table 3. Fragment assembly

Component	Amount
Vector	0.05 pmols
PCR products (for each fragment)	0.05 pmols
2X NEBuilder HiFi DNA Assembly Master Mix	10 µL
H_2O	10-x µL*
Total volume	20 µL

*x = total volume of fragments (including vector)

Fragment Preparation:

1. The reaction conditions listed in Table 2 were used to amplify fragments with designed mutations using the following primer pairs: *lacZ*-F1/R1, *lacZ*-F2/R2, *lacZ*-F3/R3, *lacZ*-F4/R4 and T7-For/T7-Rev.
2. Following PCR, 1µL of DpnI was added to each tube and incubated at 37°C for an additional 30 minutes.
3. After DpnI treatment, all products were cleaned up using Qiagen QIAquick™ PCR purification columns.

Fragment Assembly:

1. Concentration of the fragments was determined by Nanodrop™ instrument or estimated by agarose gel electrophoresis according to the specifications listed in Table 3.
2. The 2X NEBuilder HiFi DNA Assembly Master Mix was thawed at room temperature.
3. The reaction was incubated at 50°C in a thermocycler for 1 hour.
4. 2µL of the reaction was transformed into 50µL of NEB 5-alpha Competent *E. coli* (High Efficiency), plated on LB-Amp plates, and incubated overnight at 37°C.

RESULTS:

Transformation resulted in several thousand colonies. Ten colonies were screened by EcoRI-HF restriction enzyme digestion, eight of which contained the desired mutations (data not shown).

SUMMARY

These results demonstrate the versatility of the NEBuilder HiFi DNA Assembly Master Mix in multiple site-directed mutagenesis. In this example, three mutations were introduced into the *lacZ* gene simultaneously. Four overlapping PCR amplicons were assembled with a linearized vector in one step. Resulting colonies were screened by sequencing, 80% of which contained the desired mutations. This represents a substantial improvement over earlier methods of multi-site mutagenesis. Whereas previously, one may have had to create mutations sequentially, leading to a significant increase in the length of the experiment, SDM using NEBuilder HiFi DNA Assembly Master Mix can be done in one step and in much less time. In conclusion, the NEBuilder HiFi DNA Assembly Master Mix represents a substantial improvement over traditional methods, specifically in time savings, ease-of-use, and cost.

US ONLY

Description	Size	Cat. No.
NEBuilder HiFi DNA Assembly Master Mix	10 rxns	102877-804
NEBuilder HiFi DNA Assembly Master Mix	50 rxns	102877-802
NEBuilder HiFi DNA Assembly Master Mix	250 rxns	102877-806

Not available in Canada.

X-Resin DNA Challenge

Setting a New Standard in Sample Delivery

ABSTRACT

The high cost of rare biological samples, coupled with the increasing sensitivity of today's molecular methods, demands high precision pipetting by laboratory technicians[2]. Even minor variations in sample delivery can impact data quality for expression analysis[1] and next generation sequencing[3] technologies. For a number of years, pipette tip manufacturers have tried to market the efficiency of their tips for sample delivery through gravimetric studies using water, viscous dye comparisons, and even high-resolution photos showing "smoothness" of the inner tip wall. These methods, however, fail to provide quantitative assessment of tip efficiency for delivery of molecules in a given substrate. In essence, are sample molecules left behind in tips due to inherent retention of plastic?

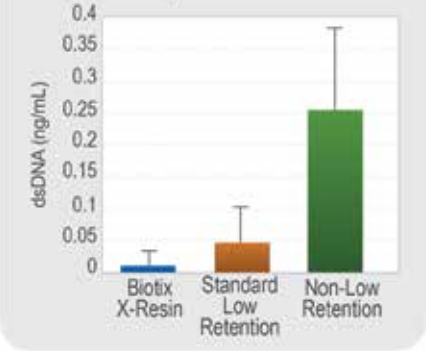
In an study performed by MRIGlobal*, a major U.S. independent, non-profit research institute, Biotix tips with X-Resin technology proved to be the most effective in preventing DNA sample loss during pipetting as compared to leading pipette tip brands for standard low retention and standard non-low retention.

METHODS

Human DNA diluted to 20µg/mL was used in the DNA challenge. The DNA solution was labeled with fluorescent dye. One hundred microliters of the fluorescent DNA solution was drawn up and down with the pipettor three full times, with a final dispense back to the original tube. One hundred microliters of molecular grade dH_2O was drawn up and down three times in the tip and dispensed into a fresh 0.5mL tube. This procedure was repeated in triplicate for each of the three types of pipette tips. DNA solutions were analyzed on the Qubit® 2.0 Fluorometer for residual fluorescent signal associated with retention of DNA solutions on the pipette tip. A reading of "Too Low" indicates less than 0.010µg/mL dsDNA detected by the fluorometer.

* More information on MRIGlobal is available at www.MRIGlobal.org

Figure: Graph of Residual DNA Carryover on Tips



RESULTS

There was a distinctive and measurable difference in sample loss due to residual DNA solution left in the tips following dispensing of the sample. Loss of sample due to residual DNA left was 0.25%, 0.71%, and 1.75% for Biotix X-Resin, Competitor A's standard low retention, and Competitor B's non-low retention tips, respectively. Biotix X-Resin tips demonstrated the best consistency and efficiency of DNA sample delivery among the three types of tips.

References

1. *Applied Biosystems, "User Bulletin #2", ABI Prism™ 7700 Sequence Detection System, Oct. 1, 2001, p.3.*
2. *Curry, D., MHale, C., and Smith, M., "Factors Influencing Real-Time RT-PCR Results: Application of Real-Time RT-PCR for the Detection of Leukemia Translocations", Molecular Biology Today, (2002) 3: 81.*
3. *Illumina, Inc., "Liquid Handling", TruSeq™ Sample Preparation Best Practices and Troubleshooting Guide, June 2011, p. 4*

Residual DNA Carryover

Sample	5µL	10µL	Mean(µg/mL)	SD
Negative Control	Too Low	Too Low	Too Low	Too Low
DNA 20 µg/mL	22.000	11.000	16.500	7.778
Biotix X-Resin – 1	0.036	0.047	0.042	0.008
Biotix X-Resin – 2	Too Low	Too Low	Too Low	Too Low
Biotix X-Resin – 3	Too Low	Too Low	Too Low	Too Low
Standard low retention – 1	0.119	0.115	0.117	0.003
Standard low retention – 2	Too Low	Too Low	Too Low	Too Low
Standard low retention – 3	0.028	0.426	0.035	0.01
Non-low retention – 1	0.13	0.448	0.289	0.225
Non-low retention – 2	0.367	0.203	0.285	0.116
Non-low retention – 3	0.205	0.185	0.195	0.014

To learn more about X-Resin, visit the redesigned Biotix page at vwr.com/biotix.

Reduced Time to Detect *Listeria* and *L. monocytogenes* in Food and Environmental Samples Using Molecular Diagnostics

DuPont Nutrition & Health has collaborated with FoodChek™ Systems Inc. to develop a new combined testing method for detecting *Listeria* species and *L. monocytogenes* from food and environmental samples. This protocol includes a 20–26 hour, single-stage enrichment in FoodChek's Actero™ *Listeria* Enrichment Media followed by automated processing with the DuPont™ BAX® System real-time PCR assays.

This new protocol was evaluated in a series of validation-level studies by FoodChek™ Laboratories on a variety of foods, including frankfurters, fresh bagged spinach, queso fresco, frozen cooked shrimp, and smoked salmon, and on environmental surfaces including stainless steel, plastic, and sealed concrete. Independent laboratory validation studies were performed for queso fresco and stainless steel samples. The results of these studies have been approved by the AOAC Research Institute (AOAC-RI) for validation as Performance Testing Methods #081401 (for *Listeria* species) and Performance Testing Methods #121402 (for *L. monocytogenes*).

Sample Preparation

Strains of *Listeria* spp. and *L. monocytogenes* were selected from FoodChek Laboratories Inc. Collection to artificially inoculate environmental surfaces and food matrices. One strain of *Pseudomonas aeruginosa* and one strain of *Enterococcus faecalis* were also selected to represent competing flora for some environmental samples. A pure culture of each *Listeria* strain was grown overnight in TSB-YE at 35°C, then diluted in either PBS or 10% non-fat dry milk to levels expected to produce fractional positive results. The selected competing flora strains were diluted to a level approximately 10-fold higher than the *Listeria* strain dilutions.

For environmental surfaces, decontaminated surfaces of 100cm^2 were spread evenly with 250µL of mixed culture of the target and competitor bacteria and allowed to dry at room temperature for 18–20 hours. Each surface was then swabbed with a sponge pre-moistened with 10mL D/E neutralizing broth and held at room temperature for at least two hours before testing.

For frankfurters, spinach, queso fresco, and smoked salmon, samples were artificially inoculated with the appropriate level of the designated *L. monocytogenes* strain, then held at 2–8 °C for 48–72 hours prior to testing. For frozen cooked shrimp, samples were artificially inoculated with the appropriate level of the designated *L. monocytogenes* strain, then held at -20°C for 14 days prior to testing.

Sample Enrichment

For the BAX System method, environmental sponges were stomached for 30 seconds with 90mL of 35°C Actero *Listeria* Enrichment Media and incubated at 35°C for 20–24 hours. Frankfurter samples (125g) were stomached for 30 seconds with 750mL of 35°C Actero *Listeria* Enrichment Media and incubated at 35°C for 26–28 hours. Spinach, queso fresco, shrimp, and smoked salmon samples (25g) were stomached for 30 seconds with 150mL of 35°C Actero *Listeria* Enrichment Media and incubated at 35°C for 22–24 hours.
For the USDA-FSIS reference method,

Table 1. BAX® System Method vs Reference Method Results for Genus *Listeria* (Environmental Samples)

Matrix	CFU / 100 cm^2	N	BAX System Method			USDA FSIS MLG 8.09 Method				
			X	PODC	95%CI	X	PODR	95%CI	dPODC	95%CI
Stainless Steel	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	5.0	20	16	0.80	(0.58, 0.92)	10	0.50	(0.30, 0.70)	0.30	(0.00, 0.53)
	63.0	5	5	1.00	(0.57, 1.00)	4	0.80	(0.38, 0.96)	0.20	(-0.26, 0.62)
Stainless Steel (Ind. Lab)	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	90.0	20	8	0.40	(0.22, 0.61)	5	0.25	(0.11, 0.47)	0.15	(-0.13, 0.40)
	220.0	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Plastic	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	4.8	20	10	0.50	(0.3, 0.7)	5	0.25	(0.11, 0.47)	0.25	(-0.05, 0.49)
	58.0	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Sealed concrete	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	4.3	20	11	0.55	(0.34, 0.74)	4	0.20	(0.08, 0.42)	0.35	(0.05, 0.58)
	22.0	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

Table 2. BAX® System Method vs Reference Method Results for Genus *Listeria* (Food Samples)

Matrix	CFU / 100 cm²	N	BAX System Method			USDA FSIS MLG 8.09 Method				
			X	PODC	95%CI	X	PODR	95%CI	dPODC	95%CI
Frankfurters	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	0.3	20	14	0.70	(0.48, 0.85)	7	0.35	(0.18, 0.57)	0.35	(0.04, 0.58)
	3.9	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Fresh Bagged Spinach	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	1.6	20	16	0.80	(0.58, 0.92)	15	0.75	(0.53, 0.89)	0.05	(-0.21, 0.30)
	1000.0	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Queso Fresco	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	0.4	20	20	1.00	(0.84, 1.00)	7	0.35	(0.18, 0.57)	0.65	(0.38, 0.82)
	13.9	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Queso Fresco (Ind. Lab)	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	0.6	20	10	0.50	(0.30, 0.70)	8	0.40	(0.22, 0.61)	0.10	(-0.19, 0.37)
	3.6	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Frozen Cooked Shrimp	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	0.7	20	12	0.60	(0.39, 0.78)	11	0.55	(0.34, 0.74)	0.05	(-0.24, 0.33)
	5.3	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Smoked Salmon	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	1.3	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(-0.27, 0.27)
	13.9	5	5	1.00	(0.57, 1.00)	5	1.0	(0.57, 1.00)	0.00	(-0.43, 0.43)

Table 3. Method Results POD Environmental Surfaces — Candiate Method Compared to Reference Culture Method

Sample Type	Listeria CFU/Sample	Samples	BAX System Method			Reference Method*			$dPOD_c$	95%CI
			Pos	POD_c	95%CI	Pos	POD_R	95%CI		
Stainless Steel	63	5	0	1.00	(0.57, 1.0)	5	1.00	(0.57, 1.0)	0.00	(-0.45, 0.43)
	5.0	20	16	0.80	(0.58, 0.92)	10	0.50	(0.30, 0.70)	0.35	(0.00, 0.53)
	0.0	5	5	0	(0, 0.43)	0	0	(0, 0.43)	0.00	(-0.45, 0.45)
Stainless Steel (Independent Lab)	220	5	5	1.00	(0.57, 1.0)	5	1.00	(0.57, 1.0)	0.15	(-0.43, 0.43)
	90	20	9	0.40	(0.22, 0.61)	5	0.25	(0.11, 0.47)	0.00	(-0.09, 0.45)
	0.0	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0.00	(-0.43, 0.43)
Plastic	65	5	5	1.00	(0.57, 1.0)	5	1.00	(0.57, 1.0)	0.00	(-0.43, 0.43)
	4.0	20	8	0.40	(0.22, 0.61)	4	0.20	(0.08, 0.42)	0.20	(-0.08, 0.44)
	0.0	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0.20	(-0.43, 0.43)
Concrete	36	5	5	10	(0.57, 1.0)	5	1	(0.57, 1.0)	0.00	(-0.45, 0.45)
	4.8	20	12	0.60	(0.39, 0.78)	10	0.5	(0.30, 070)	0.10	(-0.19, 0.37)
	0.0	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0.00	(-0.43, 0.43)

environmental sponges were stomached for two minutes with 225mL of 30°C UVM and incubated at 30°C for 24 hours. Frankfurter samples (125g) were stomached for two minutes with 1125mL of 30°C UVM and incubated at 30°C for 24 hours. For both environmental sponges and frankfurters, 100µL aliquot of the primary UVM enrichment was transferred to 9.9mL of MOPS-BLEB and then incubated at 35°C for an additional 24 hours.

For the FDA-BAM reference method, spinach, queso fresco, shrimp, and smoked salmon samples (25g) were stomached for two minutes with 225mL of 30°C BLEB without selective supplements and incubated at 30°C for four hours. Selective supplements acriflavin, nalidixic acid, and cycloheximide were then added, and the samples were incubated at 30°C for a total time of 48 hours.

Test Methods

For all samples evaluated with the BAX System method, lysis reagent was prepared by adding 150µL protease and 200µL of lysing agent to 2–12mL of lysis buffer. For each sample, 5µL enriched sample was added to 200µL prepared lysis reagent in cluster tubes. Tubes were heated for 30 minutes at 55°C and 10 minutes at 95°C, and then cooled for at least 5 minutes at 4°C. PCR tablets were hydrated with 30µL lysate and a full process was run in the BAX System Q7 instrument. All results obtained with the BAX System method were confirmed using the appropriate reference method.

For the USDA-FSIS reference method, a 10µL aliquot of each frankfurter sample enrichment was streaked onto MOX agar plates. The plates were incubated at 35°C for 24–48 hours, then checked for typical

Table 4. BAX® System Method vs Reference Method Results for *L. monocytogenes* (Food Samples)

Sample Type	MPN, CFU/Sample	N	BAX System Method X	BAX System Method PODC	BAX System Method 95%CI	Reference Method* X	Reference Method* PODR	Reference Method* 95%CI	dPODC	95%CI
Frankfurters	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	0.3	20	14	0.70	(0.48, 0.85)	7	0.35	(0.18, 0.57)	0.35	(0.04, 0.58)
	3.9	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Fresh Bagged Spinach	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	1.6	20	15	0.75	(0.53, 0.89)	15	0.75	(0.53, 0.89)	0.00	(-0.26, 0.26)
	1000.0	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Queso Fresco	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	0.4	20	19	0.95	(0.76, 0.99)	6	0.30	(0.15, 0.52)	0.65	(0.36, 0.81)
	13.9	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Queso Fresco (Ind. Lab)	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	0.6	20	7	0.35	(0.18, 0.57)	5	0.25	(0.11, 0.47)	0.10	(-0.19, 0.36)
	3.6	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Frozen Cooked Shrimp	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	0.7	20	11	0.55	(0.34, 0.74)	16	0.80	(0.58, 0.92)	-0.25	(-0.49, 0.04)
	5.3	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Cold Smoked Salmon	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	1.3	20	10	0.50	(0.30, 0.70)	10	0.50	(0.30, 0.70)	0.00	(-0.28, 0.28)
	13.9	5	5	1.00	(0.57, 1.00)	5	1.0	(0.57, 1.00)	0.00	(-0.43, 0.43)

Listeria colonies. Typical colonies were confirmed using biochemical test panel *Listeria* API® system.

For the FDA-BAM reference method, all spinach, queso fresco, shrimp, and smoked salmon sample enrichments were directly streaked onto MOX agar plates after both 24 and 48 hours of enrichment. Plates were incubated at 35°C for 24 to 48 hours, then checked for typical *Listeria* colonies. Typical colonies were then picked and confirmed using biochemical test panel Listeria API system.

Results and Discussion

Probability of detection (POD) statistical model was used to evaluate the differences between the BAX System method and the reference methods. All the data are presented in summary tables of POD values, dPOD values, and confidence intervals by sample type and concentration. For most sample types evaluated, POD analysis showed no statistically significant differences between the performance of the BAX System method and the reference methods (the 95% confidence interval of the dPODs contains zero).

However, POD analysis showed a statistically superior performance for the following BAX System Real-Time PCR Assays compared to the appropriate reference method:

- Concrete and stainless steel samples for Genus *Listeria*
- Frankfurter and queso fresco samples for Genus *Listeria* and *L. monocytogenes*

Conclusions

The results of these studies demonstrate that:

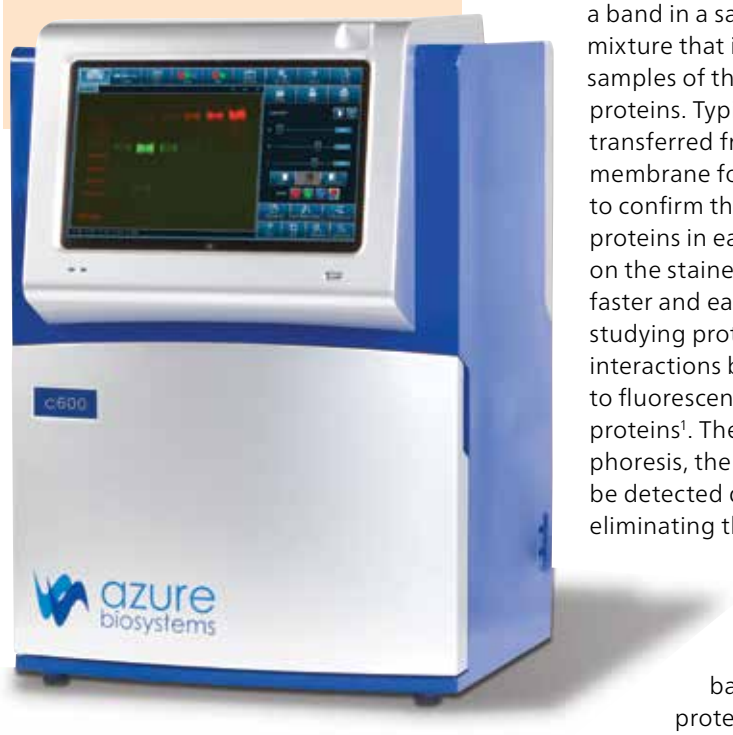
- The BAX System method using Actero *Listeria* Enrichment Media is statistically equivalent or superior to the reference culture methods for detecting *Listeria* spp. in environmental and food samples.
- The time to detect *Listeria* spp. and *L. monocytogenes* was significantly reduced when single-step enrichment in Actero *Listeria* was combined with the BAX System assay.
- The high accuracy and reliability of the method were confirmed by independent laboratory validation studies.

US ONLY *Not available in Canada.*

Contact your VWR representative for more information on the BAX System.

Imaging In-Gel Fluorescence and Stain-Free™ Gels with the Azure™ c600™

Detecting in-gel fluorescence enables the analysis of protein gels immediately after electrophoresis, without staining or Western blotting. Separation of proteins by electrophoresis is a cornerstone of protein analysis. After separation, identification of proteins typically requires several additional steps, either incubating the gel in a staining solution to stain the proteins, or transferring the proteins to a membrane for Western blotting and/or staining. Stain-Free gels incorporate compounds in the gel matrix that react with proteins upon exposure to UV light, generating fluorescent products that, with an appropriate imaging system, can be detected within the gel without further staining.



In-gel fluorescence detection also presents a timesaving approach to electrophoretic mobility shift or "gel shift" assays. The assays, frequently used to detect protein-nucleic acid interactions[2], can also be used to detect protein-protein interactions. The technique is based on the fact that two proteins bound together in a complex will migrate differently through a native gel than will either protein alone. Binding is observed by staining the gel and detecting a band in a sample of a protein mixture that is not seen in samples of the individual proteins. Typically, proteins are transferred from the gel to a membrane for Western blotting to confirm the identities of the proteins in each band observed on the stained gel. A much faster and easier approach to studying protein-protein interactions by gel shift assays is to fluorescently label one of the proteins[1]. Then, after electrophoresis, the fluorescence can be detected directly in the gel, eliminating the need to stain the gel or to conduct a Western blot to identify new bands containing the protein of interest.

In this technical note, we present the use of the c600 to image a fluorescent gel-shift assay. Fluorescently labeled ubiquitin was incubated with the E1 ubiquitin-activating and E2 ubiquitin-conjugating enzymes, and the binding reactions analyzed by gel electrophoresis. First, free and bound ubiquitin were detected by capturing the in-gel fluorescence of the fluorescein-labeled ubiquitin. Then, total protein was detected fluorescently, without staining the gel or even removing it from the imager.

METHODS

A series of incubations were conducted combining fluorescein-labeled ubiquitin and the E1 and E2 proteins. The binding reactions were quenched after 0.5, 1, 2, 4, 8, 16, 30, and 60 minutes by the addition of non-reducing SDS buffer. The samples were then loaded on a stain-free precast gel and electrophoresis was conducted under non-denaturing conditions. The samples in each lane contained approximately 200ng of fluorescein-labeled ubiquitin.

After electrophoresis, fluorescein-labeled ubiquitin in the gel was detected by imaging in the Azure c600, using the epi blue light source and filter in the gel acquisition mode. A 6–second exposure was captured.

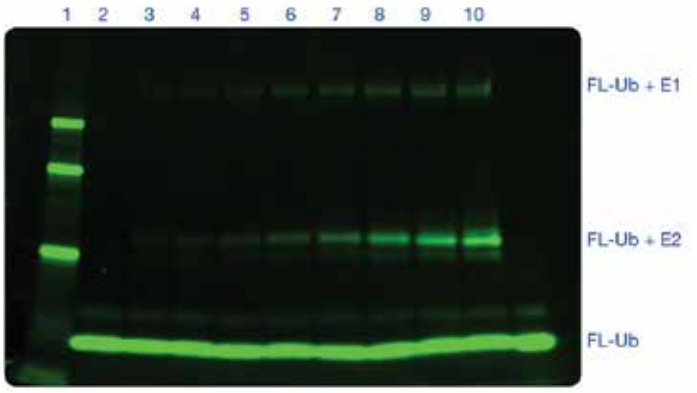


Figure 1. Fluorescent gel-shift assay using fluorescein-labeled ubiquitin (FL-Ub). FL-Ub, E1, and E2 were incubated for the indicated times and the reactions quenched with non-reducing SDS buffer. E1 and E2 bands with increasing fluorescence indicate covalent binding of fluorescent-labeled ubiquitin to E1 and E2. Fluorescein fluorescence was detected in the Azure c600 using excitation at 47nm and emission filter at 497nm. Lane 1: MW marker; lane 2: 0 min; lane 3: 0.5 min; lane 4: 1 min, lane 5: 2 min; lane 6: 4 min; lane 7: 8 min; lane 8: 16 min; lane 9: 30 min; lane 10: 60 min.

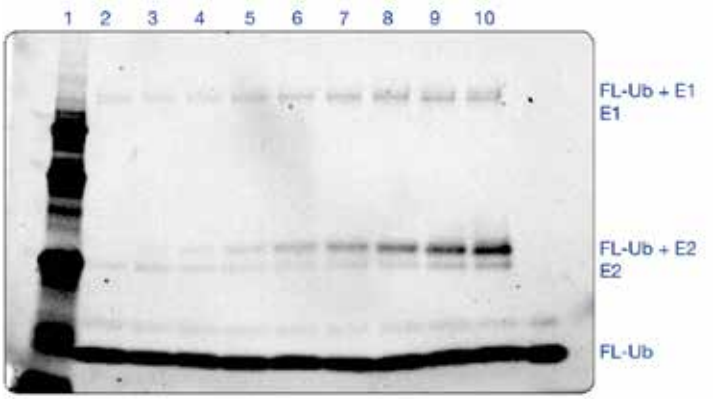


Figure 2. In-gel fluorescence imaging of total protein. The gel in Figure 1 was cross-linked for 5 minutes with the UV transilluminator in the Azure c600 imaging system, and then total protein detected in the UV365 channel. Lane 1: MW marker; lane 2: 0 min; lane 3: 0.5 min; lane 4: 1 min, lane 5: 2 min; lane 6: 4 min; lane 7: 8 min; lane 8: 16 min; lane 9: 30 min; lane 10: 60 min.

After the fluorescein image was captured, total protein was imaged per instructions for stain-free gels. Briefly, using the UV transilluminator of the Azure c600, the gel was exposed to UV light at 302nm for five minutes to cross-link trihalo compounds within the gel to tryptophan residues in proteins. Fluorescent protein bands were then detected by imaging the gel using the UV 365nm light source and filter in the gel acquisition mode. A 10-second exposure was captured.

RESULTS

The binding reactions were loaded onto a gel and proteins separated by gel electrophoresis under non-denaturing conditions. In-gel fluorescent imaging of the gel reveals free fluorescein-labeled ubiquitin and a time-dependent increase in both E1-ubiquitin and E2-ubiqitin complexes (Figure 1).

After detecting the in-gel fluorescence of fluorescein, the gel was left in the Azure c600 instrument and exposed to UV light at 302nm to crosslink the trihalo compounds in the gel to tryptophan residues in the proteins.

Once crosslinking was complete, the gel was imaged to detect total protein. The presence of free E1 and E2 proteins in the reaction mixtures can be detected, as well as the E1-ubiquitin and E2-ubiquitin complexes that were seen when the gel was imaged for fluorescein (Figure 2).

CONCLUSIONS

The Azure c600 serves as a "one-stop shop" for fluorescent gel-shift assays. Using stain-free gels, both fluorescently labeled protein and total protein can be imaged without removing the gel from the imaging system. The resulting images can be overlaid to reveal which bands contain the fluorescently labeled protein, alleviating the need to stain the gel or conduct time-consuming Western blots.

Acknowledgements

Azure would like to thank Chris Balak, Dr. Jesse Hunter, and Dr. Lisa Baumbach-Reardon of the Integrated Cancer Genomics Division of the Translational Genomics Research Institute (TGen) for permission to use these data.

References

1. *Park, Sang-Hyun, and Ronald T. Raines. "Fluorescence gel retardation assay to detect protein-protein interactions." Protein-Protein Interactions. Humana Press, 2004.155-159.*
2. *Rio, Donald C. "Electrophoretic mobility shift assays for RNA-protein complexes." Cold Spring Harb Protoc 2014. doi:10.1101/pdb.prot080721*

Description	Cat. No.
Azure c600 Ultimate Western Blot Imaging System	**10147-214**

Even the Smallest Thing May Have a Big Impact

Western blotting is a staple technique that has long been used for qualitative protein analysis to confirm protein presence and to estimate protein amount. But how has Western blotting evolved over time? Could seemingly small changes to protocols, reagents, or workflow have a significant impact on the reliability of your results, speed of analysis, or longevity of signals? We believe so.

Since the early 1990's, Life Sciences from GE Healthcare has been committed to continuous development of Amersham™ ECL™ Western blotting detection systems. As a result, we have advanced this protein analysis technique from confirmatory to quantitative and made the process increasingly stable and robust.

Whether you need quick confirmation or in-depth characterization of your protein, your protein is scarce or abundant, or you run Western blots daily or occasionally, you need consistency and reliability. The success is all in the details— in careful experimental design and in the selection of the right detection system for the task.

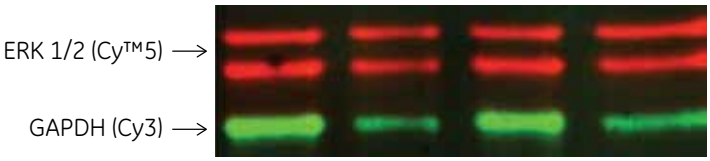
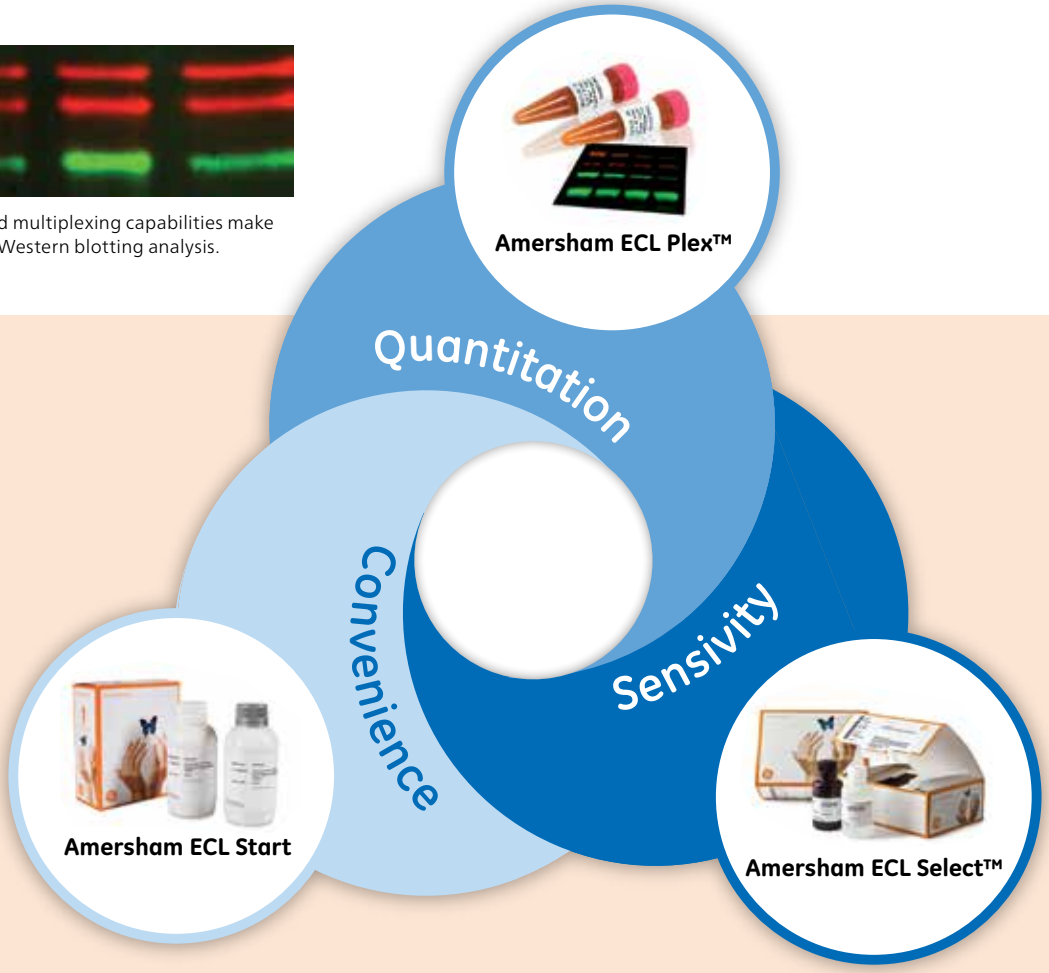


Figure 1. Stable signals, high reproducibility, and multiplexing capabilities make Amersham ECL Plex well suited for quantitative Western blotting analysis.



Primary Ab dilution	Secondary Ab dilution	10.0 5.0 2.5 1.2 0.6 0.3 µg
1:3000	1:100 000	
1:5000	1:100 000	
1:10 000	1:100 000	
1:15 000	1:100 000	

Figure 2. Amersham ECL Select provides high sensitivity using a wide dilution range of primary antibodies.

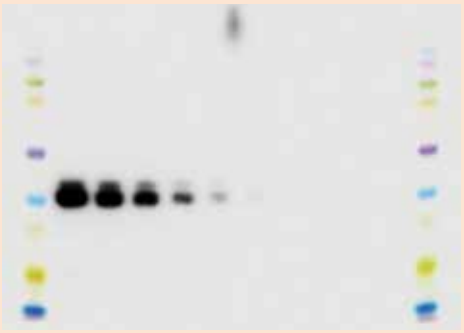


Figure 3. Western blotting detection using Amersham ECL Start, showing resulting blots from ERK 1/2 in NIH/3T3 lysate. The high signal intensity fully utilizes the advantages of CCD camera-based imaging equipment.

Change to Fluorescent Western Blotting and Spend 50% Less Time in the Lab to Obtain Your Quantitative Data

When reliable quantitation is a priority, the fluorescent Amersham ECL Plex detection reagents generate stable signals, high reproducibility, and multiplexing capabilities. These reagents allow convenient normalization of target proteins against a housekeeping protein or total protein in just one step. Without the need to strip and re-probe, quantitative data can be reliably generated up to 50% faster. Therefore, fluorescence-based Amersham ECL Plex is excellent choice for quantitative Western blotting applications.

100 More Western blot Experiments from Your Precious Antibodies

When high sensitivity is critical, we recommend Amersham ECL Select, the most sensitive chemiluminescent detection reagent in the Amersham ECL product family. Due to the high sensitivity of Amersham ECL Select, it is possible to use highly diluted primary antibodies as probes on your blotted membranes. This both reduces cost and avoids levels of background signals that may quench the weaker, specific interactions of interest.

Change to Amersham ECL Start and Spend Less Time Worrying about Expiry Dates

Amersham ECL Start is a chemiluminescent detection reagent recommended for confirmatory analysis and detection of medium- to highly-expressed proteins in cells and tissue and of proteins from various recombinant expression systems. With a shelf life of up to 18 months, Amersham ECL Start adds flexibility if you run Western blots infrequently and is suitable for most immunoblotting experiments, so select it when you need to progress your research without delay.

Size	Cat. No.
Amersham ECL Start Western Blotting Detection Reagent	
200 mL for 2000 cm^2 Membrane	**10662-310**
400 mL for 4000 cm^2 Membrane	**10662-312**
Amersham ECL Select Western Blotting Detection Reagent	
100 mL for 1000 cm^2 Membrane	**89233-310**
Amersham ECL Plex Combination Pack	
Hybond-ECL 10 x 10 cm for 1000 cm^2 Membrane	**95040-084**
Hybond-LFP 20 x 20 cm for 1000 cm^2 Membrane	**95040-086**

Comparison of Two Colorimetric Assays in the Detection of Cytotoxicity in Fibroblasts Initiated by Apoptotic Inducers

G-Biosciences offers several colorimetric cell-based proliferation and toxicity assays that have been optimized for microplates. The assays allow for many samples to be analyzed rapidly and simultaneously using compounds that stain the cells directly or that are metabolized into colored products.

Cytoscan™ SRB assay is based on the sulforhodamine B dye (Figure 1) assay that is reported to be a standard assay of the National Cancer Institute and National Institute of Health (http://dtp.nci.nih.gov/branches/btb/ivclsp.html) (Nedel). SRB is a bright-pink aminoxanthene dye that binds to basic amino acids of cellular proteins under mild acidic conditions, and dissociates under basic conditions[5]. SRB binding is stoichiometric and the amount of dye extracted from stained cells is directly proportional to the total protein mass and therefore correlated to cell number[3-5].

Cytoscan WST-1 assay is based on the metabolism of the water soluble tetrazolium salt (WST) (Figure 2) 4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate.

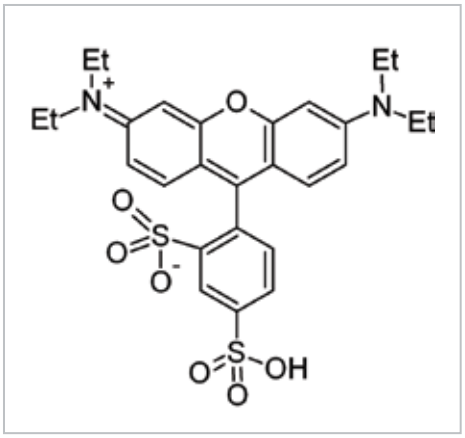


Figure 1. Structure of Sulforhodamine B (SRB).

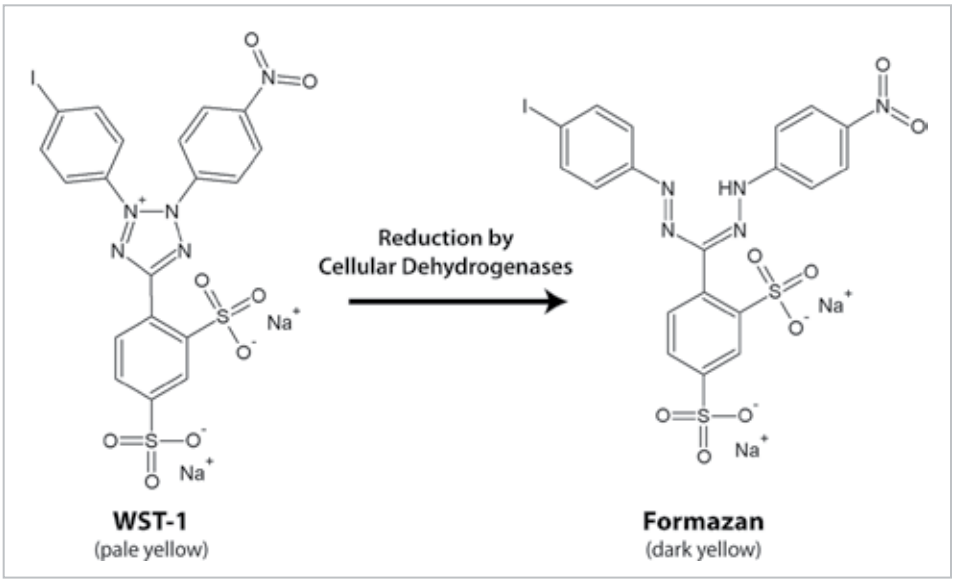


Figure 2. Reduction of WST-1 to a colored formazan by cellular dehydrogenases.

Several other tetrazolium salts are used in cell proliferation and toxicity assays, including, but not limited to, MTT, XTT and MTS[3].

In contrast to the SRB assay, the tetrazolium salt assays are based on the metabolic reduction of the salt to a highly colored formazan end-products[1]. With WST-1, which is cell-impermeable, reduction occurs outside the cell via plasma membrane electron transport. In addition, WST-1 assays can be read directly, unlike MTT that needs a solubilization step, give a more effective signal than MTT, and decrease toxicity to cells (unlike cell-permeable MTT, and its insoluble formazan that accumulate inside cells).

Significant differences may exist in the sensitivity of Cytoscan SRB and Cytoscan WST-1 assays as they utilize different labeling systems to assay cell proliferation and toxicity.

Inducer	Concentrations (µM)
Actinomycin D	0, 0.2, 0.3, 0.5, 0.6, 0.8
Camptothecin	0, 2, 4, 6, 8, 10
Cycloheximide	0, 7, 14, 21, 28, 35
Doxorubicin	0, 0.5, 1, 2, 2.5, 3

Table 1. Apoptotic inducer concentrations used.

AIM

To compare Cytoscan SRB and Cytoscan WST-1 in their monitoring of the effects of several apoptotic inhibitors on the growth on NIH3T3 fibroblasts.

METHOD

NIH3T3 cells were seeded on 96-well plates at ~4x10^3 cells per well and allowed to adhere to the plates overnight in DMEM supplemented with FBS.

The above apoptotic inducers were prepared at the indicated concentrations in DMEM without FBS the morning of treatment (Table 1).

The growth media was aspirated from the cells and the cells were washed once in phosphate buffered saline (PBS). The apoptotic inducers were added in duplicate and the plates incubated at 37°C for 24 hours. After 24 hours the media was removed and replaced with fresh media and the appropriate assay was performed.

Cytoscan SRB Protocol

50µL fixative reagent was gently added to each well and the plates were incubated at 4°C for 1 hour. The cells were washed with deionized water and the plates were dried at 50°C for 30

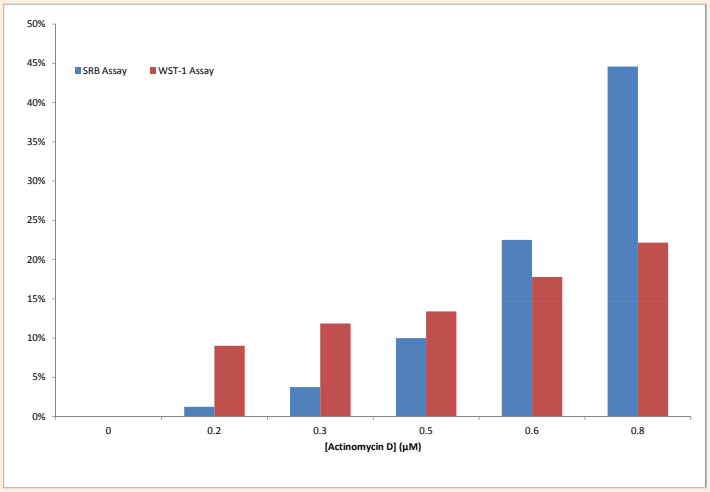


Figure3. Effects of actinomycin D on fibroblast cells as monitored by Cytoscan SRB and Cytoscan WST-1 assays.

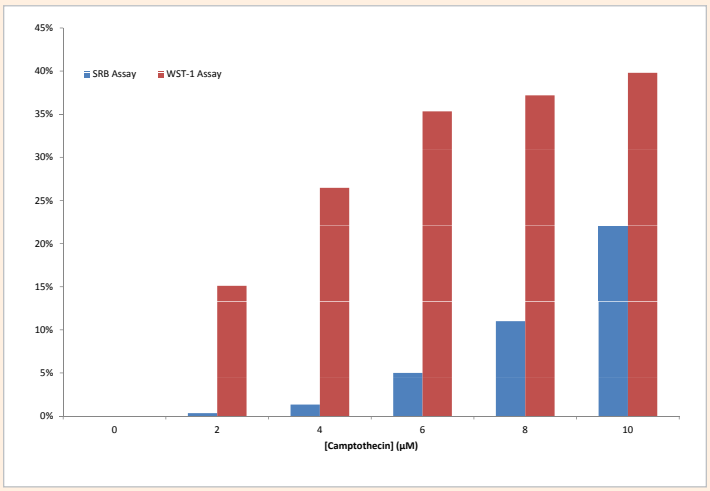


Figure 4. Effects of camptothecin on fibroblast cells as monitored by Cytoscan SRB and Cytoscan WST-1 assays.

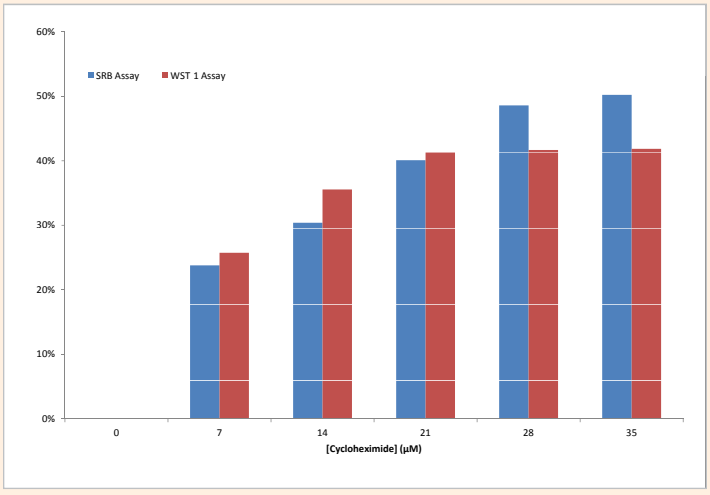


Figure 5. Effects of cycloheximide on fibroblast cells as monitored by Cytoscan SRB and Cytoscan WST-1 assays.

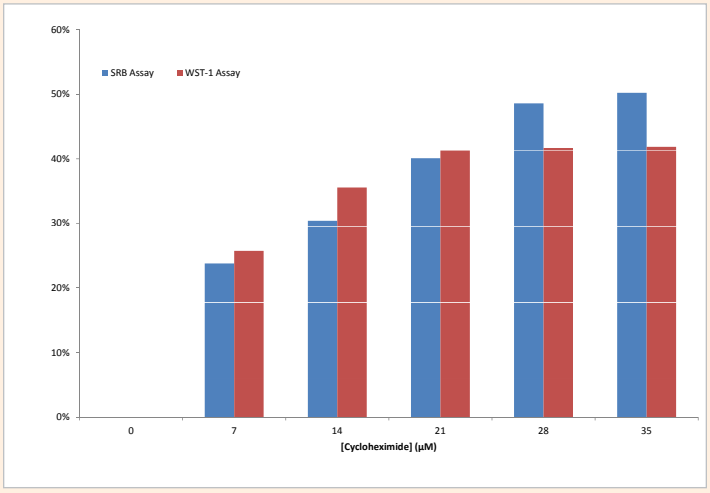


Figure 6. Effects of doxorubicin on fibroblast cells as monitored by Cytoscan SRB and Cytoscan WST-1 assays.

minutes. 100µL SRB dye solution was added to the cells and incubated at room temperature in the dark for 30 minutes. The plates were then washed as before using the 1X dye wash solution. The plates were dried and then 200µL SRB solubilization buffer was added to each well to dissolve the bound SRB dye. The absorbance of the bound dye was measured at 570nm.

Cytoscan WST-1 Protocol

10µL WST-1/CytoScan Electron Carrier (CEC) Assay dye solution was added to each well and the plates were gently shaken to mix chemicals with the medium. The plates were incubated in the cell culture incubator for 2 hours. The amount of formazan produced was measured at 440nm.

The percentage cytotoxicity for each assay was calculated as shown below:

$$\% \text{ Cytotoxicity} = 100 \times \frac{(\text{Control} - \text{Experimental})}{\text{Cell Control}}$$

RESULTS AND DISCUSSION

Actinomycin D is a class of polypeptide antibiotic isolated from the *Steptomyces* soil bacteria. Actinomycin D inhibits transcription by binding to DNA at the transcription initiation complex and therefore preventing elongation by the RNA polymerase. The effects on the induction of apoptosis vary by two fold between the SRB and WST-1 assays (Figure 3). The SRB assay showing almost 50% cytotoxicity compared to only 22% with the WST-1 assay at 0.8µM.

Camptothecin is a cytotoxic quinoline alkaloid that inhibits the DNA enzyme topoisomerase I (Topo I). The effects of camptothecin varied between the SRB and WST-1 assays with the WST-1 assay showing a greater sensitivity compared to SRB (Figure 4). The WST-1 assay was able to detect 15% cytotoxicity with 2µM camptothecin, whereas the SRB assay was only detecting similar levels at >8µM.

Cycloheximide is an inhibitor of protein synthesis by interfering with the translocation step (movement of two tRNA molecules and mRNA on ribosome) in protein synthesis, thus blocking translational elongation. Both the SRB and WST-1 assays showed similar profiles for cytotoxicity, however the WST-1 assay appears to plateau earlier than the SRB assay (Figure 5).

Doxorubicin is an anthracycline antibiotic that intercalates DNA, inhibiting the unwinding of DNA by topoisomerase II. Both the SRB and WST-1 showed similar profiles for cytotoxicity with little variation (Figure 6).

DISCUSSION

The above investigation is a preliminary investigation into the differences that may result from two different technologies routinely used to monitor cell proliferation and toxicity. Further work needs to be undertaken to delineate the reasons for the differences seen with induction of apoptosis with actinomycin D and camptothecin and to some extent cycloheximide.Both Cytoscan SRB and Cytoscan WST-1 were both fully capable of detecting cytotoxicity as a result of apoptotic inducers used in a dose dependent manner.

Researchers have the choice of several colorimetric cell proliferation and toxicity assays, however from our data and reported data, researchers should be cautious about comparing data generated by different assays reportedly showing the same effect.

Citations

Cytoscan SRB

1. *Boyerinas, B et al (2012) IJC. 130:1787*
2. *Cross, C. et al (2015) Biomed Res Int 25:7090*
3. *Haque, F. et al (2012) Nanotoday. 7:245*
4. *Tolstikov, V. et al (2014) PLOS. DOI: 10.1371/journal. pone.0114019*
5. *Xiao, Y. et al (2013) Neoplasia 15:1151*
6. *Xiao, Y. et al (2013) Neoplasia 15:1137*
7. *Zhao, Y. et al (2012) ACS Nano. 6:8684*

Cytoscan WST-1

1. *Chen, Y. et al (2013) Langmuir. 29:3721*
2. *Galas, R.J. and Liu, J. (2014) J. Cell. Biochem. 115:111*
3. *Hagmann, W. et al (2011) Cancers. 3:106*
4. *Hsieh, C. et al (2014) Food Funct. 5: 2494*
5. *Kuo, C. et al (2013) Allergy. 68:870*
6. *Neelofar, K. et al (2011) Can. J. Microbiol. 57:204*
7. *Li, H. et al (2009) Mol Cancer Ther 8:3255*
8. *Schweigmann, H. et al (2014) J. Steroid Biochem. Mol. Biol. 143:259*

References

1. *Berridge, M.V. et al (1996) Bioschemica. 4:14*
2. *Berridge, M.V. et al (2005) Biotech. Ann. Rev. 11:127*
3. *Nedel, F. et al (2011) Int. Endod. J. 44:59*
4. *Papazisis, K.T. et al (1997(J. Immunol. Meth. 208:151*
5. *Vichai, V. and Kirtikara, K. (2006) Nature protocols. 1:1112*

Description	Size	Cat. No.
CytoScan SRB	1000 Assays	**95029-190**
CytoScan WST-1	500 Assays	**95029-212**
Apoptotic Inducers		
Actinomycin D [10mM]	50 µL	**82021-596**
Camptothecin [2mM]	1 mL	**82021-598**
Cycloheximide [100mM]	1 mL	**82021-600**
Doxorubicin [2mM]	50 µL	**82021-604**

InstantBlue™ Protein Stain

C.B.S. SCIENTIFIC COMPANY, INC

1 liter bottle of InstantBlue,
Cat. No. 95045-070

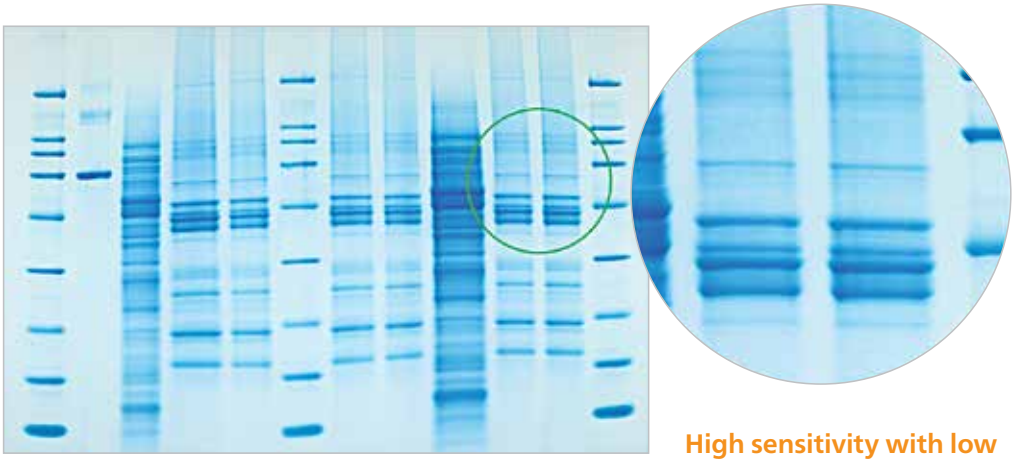
THE FASTEST, EASIEST AND BEST PROTEIN GEL STAIN

Easy to Use: Remove SDS, fix and stain in ONE STEP. No destain is needed.

Fast: Ready to use formulation with results in 10 to 30 minutes

Sensitive: As little as 5ng/band (BSA) of protein can be detected with low background

Safe: Non-toxic formula contains no methanol, suited for regular use and disposal



16% ClearPAGE SDS precast gel
Lanes 4,5,7,8,10,11 -Chicken lysate. Lanes 3, 9 -*E.Coli*, Lane 2-BSA, Lanes 1,6,12 - BioRad Markers

High sensitivity with low background staining

Description

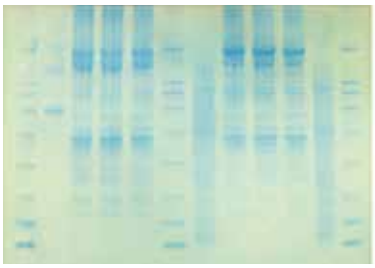
InstantBlue stain is a ready-to-use Coomassie G-250 stain that is optimal for obtaining well defined protein bands. InstantBlue is the only true single step Coomassie based gel stain available. Protein gel staining takes only 10-30 minutes because there is no need to wash, fix, microwave, or destain. InstantBlue stain contains an SDS-sequestering agent that makes it fast and easy to use. Since SDS binds stain, it normally must be removed to get good sensitivity. Often some SDS remains in the gel, which contributes to background and requires destaining. The SDS-sequestering agent removes the SDS from the gel, so no washing or destaining is necessary. Its special formulation has the benefits of high sensitivity (approximately 5-25ng of protein detected per band) and low background interference-improving the overall gel resolution. Additionally, it contains no methanol and is ideally suited for regular use and disposal.

Benefits

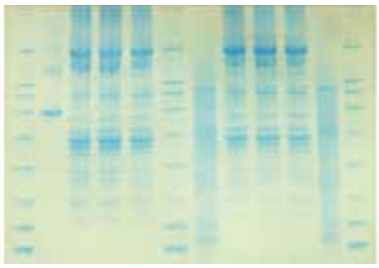
- Ultra-fast staining — all bands visible within 15 minutes.
- Single step procedure — no need to wash, fix, microwave, or destain. Just take the gel out of the cassette and place in a container with InstantBlue.
- High sensitivity — approximately 5-25ng of protein detected per band.
- Super-Low background staining — only the proteins are stained, resulting in extremely well defined

C.B.S. SCIENTIFIC COMPANY, INC

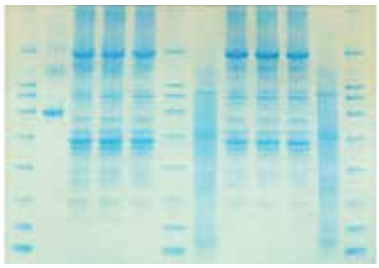
Stain Comparisons	InstantBlue™	Competitor 1	Competitor 2	Home-made
No need to wash before staining	✓	3x5 min	3x5 min	Typically 3x5 min
No need to fix before staining	✓	✓	x	x
No need to heat microwave	✓	x	✓	✓
No need to destain	✓	x	x	x
No risk of overstaining if left too long	✓	x	x	x
Non-toxic and easily disposed of	✓	✓	x	x



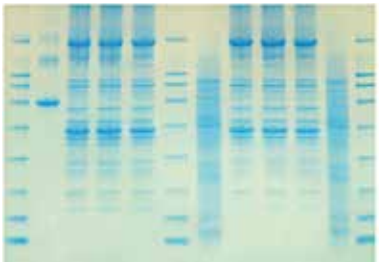
InstantBlue Stain 10 Minutes



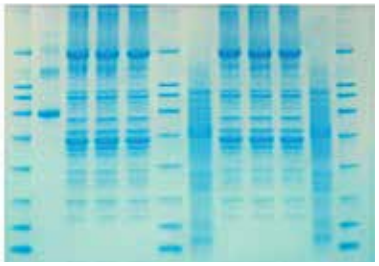
InstantBlue Stain 15 Minutes



InstantBlue Stain 30 Minutes



InstantBlue Stain 45 Minutes



InstantBlue Stain 1 Hour

16% ClearPAGE SDS precast gel
Lanes 4,5,7,8,10,11 -Chicken lysate.
Lanes 3, 9 -*E.Coli*, Lane 2- BSA, Lanes 1,6,12 - BioRad Markers

Protein mixtures electrophoresed on a 16% SDS ClearPAGE gel and stained in InstantBlue™ for 10, 15, 30, 45 and 60 minutes. Above gel images were not washed or destained.

blue bands on a highly transparent background. No possibility of over staining means that you can leave your gel in the stain indefinitely and still be able to read it.

- Quantitative — same gel-to-gel performance ideal for quantifying protein by densitometry
- Non-toxic— ideal for regular use and disposal. Can be poured down sink with no adverse environmental consequences.
- Mass Spec Compatible[1]

Procedure

1. Mix InstantBlue before use by inverting the bottle a few times.
2. Remove the gel from the gel tank and submerse the gel in enough InstantBlue to cover the gel (approximately 20mL). Place on orbital shaker.
3. Protein bands will be visible within 15 minutes. For best results leave in stain for at least 1 hour.
4. Destaining does NOT improve sensitivity, but the gels may be washed in ultrapure water for 15 minutes to remove the free stain from the gel and get clear backgrounds. The gel may be stored in ultrapure water.

Reference

1. Zhu, Z., Boobis, A.R., Edwards, R.J., Identification of estrogen-responsive proteins in MCF-7 human breast cancer cells using label-free quantitative proteomics, Proteomics 2008, 8, 1987-2005.

Description	Cat. No.
InstantBlue Stain, 1 L	**95045-070**

Corning® Offers a Complete Solution for Serum-Free Hybridoma Scale-Up and Protein Production

Monoclonal antibodies (mAb) are widely used for biochemistry, molecular biology, and as potential therapeutic candidates. The ability to generate large quantities of mAb to meet these basic research and other large-scale manufacturing needs can be challenging. With the potential increase in antibody uses as therapeutic candidates, more efficient and cost effective methods for manufacturing antibodies will be key.

Corning® hybrigro™ SF Medium is a complete, animal-free, defined medium specifically developed for serum-free growth and antibody production with a variety of hybridoma cell lines. Corning hybrigro SF medium supports this initiative by increasing hybridoma yields and production efficiency without the use of costly serum. Testing demonstrates that Corning hybrigro SF outperforms two commercially available serum-free hybridoma media, resulting in higher cell density and protein production using two different hybridoma cell lines. (Chart 1, 2)

Corning® CELLine™ Disposable Bioreactors enable the *in vitro* generation of high yields of mAb in an

easy-to-use, disposable vessel. Hybridoma cells were cultured under serum-free conditions using Corning hybrigro SF medium in the Corning CELLine disposable bioreactor and traditional cell culture flasks to demonstrate increased protein production and the concentration of product generated using this system.

The unique design of the CELLine disposable bioreactor combined with Corning hybrigro SF™ medium is ideal for users that require high yields of concentrated mAb that are produced under defined, serum-free culture conditions. (Chart 3)

Description	Size	Cat. No.
Hybrigro SF with Glutagro	500 mL	**11010-977**
CELLine 1000 System	—	**47735-592**

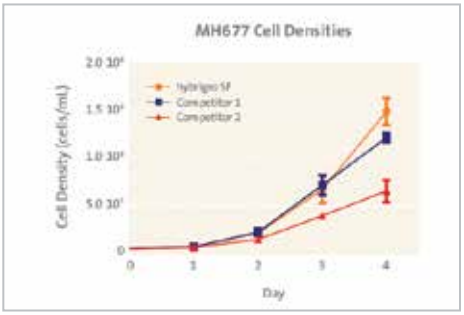


Chart 1. MH677 cells showed significantly increased cell densities by day four compared to competitor serum-free hybridoma media (n=9 ANOVA Newman-Keuls Post Test).

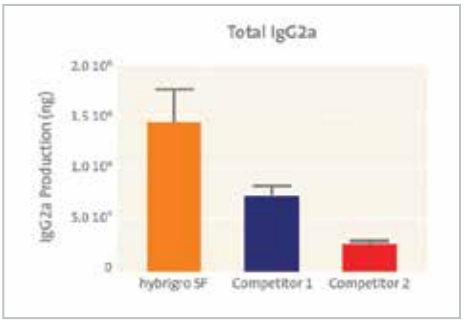


Chart 2. MH677 cultured in Corning hybrigro SF showed statistically higher levels of IgG2a production compared to both competitor serum-free media (n=9 ANOVA Newman-Keuls Post Test).

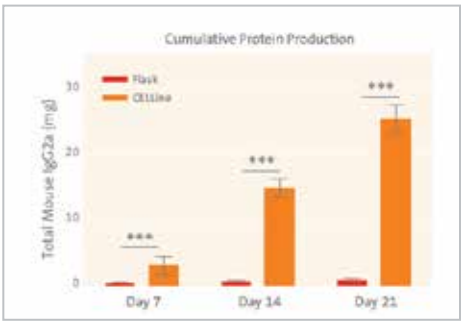


Chart 3. Cumulative IgG2a production. Significantly more IgG2a was produced in the Corning CELLine bioreactors when compared to production from T-75 flasks. Data shown with standard deviation. Unpaired T-test ***p<0.001. n=6 vessels from 3 independent studies.

Rapid Apoptosis Screening with Orflo's Moxi Flow™ Cytometer

INTRODUCTION

Cellular apoptosis is a sophisticated mechanism employed by cells to carefully control death in response to cell injury. Commonly referred to as "programmed cell death," apoptosis progresses through a systematic signaling cascade that results in characteristic, directed morphological and biochemical outputs in the cell. The overall outcome is a highly regulated cell death process that minimizes trauma to the cell's extracellular environment. At a systemic level, the critical role of apoptosis is easily highlighted through its implication in numerous pathologies[1-5]. Correspondingly, scientific research has intently focused on revealing the underlying pathways of apoptosis, defining its external triggers, and identifying therapeutic interventions. Furthermore, apoptosis has also been established as a key indicator in identifying the "biocompatibility" of pharmaceuticals and other environmental conditions for cell systems.

Concurrent with the increased interest in apoptosis monitoring, numerous assays have been created to quantify the expression of molecular targets such as phosphatidylserine (PS) externalization in the plasma membrane and caspase enzyme activation. However, these assays often require advanced technical expertise, costly instrumentation, and time-consuming techniques such as western blot, ELISA, and flow cytometry. Here, we present the Orflo Moxi Flow system as a simple, rapid, and effective alternative to monitoring cellular apoptosis via two of the most common techniques: 1.) Fluorescently-coupled Annexin V tagging of PS externalization on the plasma membrane and 2.) Fluorescent Labeled Inhibitors of Caspases (FLICA) detection of activated caspase-enzymes.

METHODS

Cell Culture

Jurkat E6-1 (ATCC) were cultured (37°C, 5% CO_2) in RPMI-1640 supplemented with 10% FBS, 1mM Sodium Pyruvate, and 10mM HEPES. For apoptosis induction, 500µL of 1mM camptothecin stock (in DMSO) was added to 10mL of culture media. 4.5mL of the Jurkat suspension was then added (30µM final camptothecin). For the negative control, DMSO was substituted for camptothecin.

PE-Annexin V Assay

Cells were labeled with Annexin V-PE (BioLegend), following Orflo's Moxi Flow Apoptosis (PE-Annexin V) and Viability (PI) Staining Protocol. After preparation, cells were run on the Moxi Flow system using the Apoptosis Assay. A separate sample was stained with Moxi Cyte Viability Kit following the protocol for using the viability assay on the Moxi Flow. For the time-course data, cells were assayed on the Moxi Flow at ten discrete time points over a ~10hr period. Three measurements were made at each time point.

SR-FLICA Caspase 3/7 Assay

Jurkat E6-1 (ATCC) cells were induced to apoptosis with 30µM camptothecin as described above. After a six hour incubation period, the cells were labeled with a sulforhodamine (SR)-FLICA Caspase-3/7 Assay Kit following the manufacturer's protocol. A separate, PE-Annexin V sample was prepared (as described above) for direct comparison purposes.

RESULTS AND DISCUSSION

The Moxi Flow is a flow cytometer with a 532nm (green) laser and 590/40nm PMT collection filter, tailored for capturing emission from Phycoerythrin (PE) and Propidium Iodide (PI). At the core of the system is a disposal flow cell cassette that precisely counts and sizes particles (Coulter Principle) and exactly measures fluidic volume. The entire test is performed on-cassette, so no fluid enters the system, obviating the need for time-consuming cleaning, disinfecting, and shutdown procedures.

Two well-characterized, early-stage markers in the cellular apoptosis progression are the activation of caspase enzymes, involved in the signaling cascade, and the translocation of the membrane phospholipid, phosphatidylserine (PS), from the inner to the outer leaflet of the plasma membrane. The data shown in Figure 1 highlight the ability of the Moxi Flow to track and quantify each event in a cell sample. Figure 1A shows the results of camptothecin-treated Jurkat cells labeled with SB-FLICA probes. The FLICA approach uses fluorophore-coupled, membrane-permeable peptide sequences that covalently bond only to activated caspase enzymes. Following a wash of the cell sample, cells with elevated levels of these activated caspases (apoptotic cells) fluoresce. The data in Figure 1A show specific labeling of caspase-3 and caspase-7

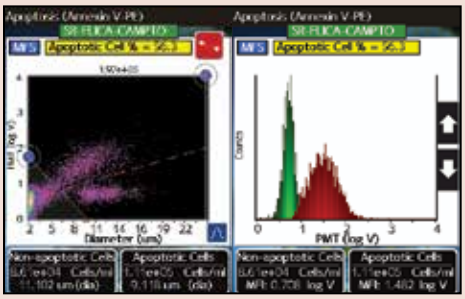


Figure 1A. Camptothecin-treated Jurkat cells labeled with SB-FLICA probes. The apoptotic cell population shows a clear separation for labeled cells.

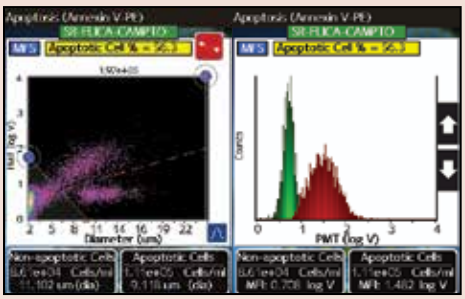


Figure 1B. Sample was targeted with a PE-conjugated Annexin V probe to show PS translocation event.

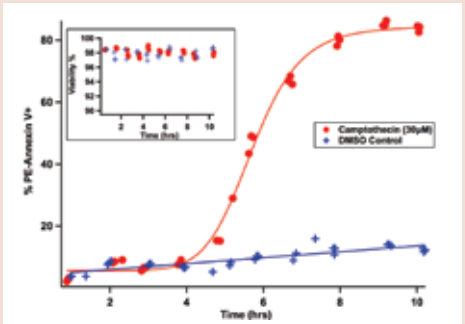


Figure 2 shows the activation kinetics over time for apoptosis induced by camptothecon.

enzymes with a clear separation in the apoptotic cell population. In Figure 1B the PS translocation event is detected on the same sample by targeting it with a PE-conjugated Annexin V probe, a cellular protein with a high-affinity for PS. Because the Annexin V probe is cell-impermeable, only cells with apoptosis-induced exposure of PS, or cells with compromised membranes (necrotic cells), are labeled with this probe. As can be seen in Figure 1B, this Annexin V+ population readily separates from the Annexin V- cell group. The percentages of the two methods (SR-FLICA and PE-Annexin V), correlate well (56% and 62% respectively) as would be expected. As a final step, a quick (5min, PI) viability assay was used to determine the sub-population/percentage of necrotic cells that contributed to the apoptotic positive populations in both assays. The early-stage apoptotic cell percentage could then be readily calculated through a simple subtraction of the PI+ cell percentage from the Annexin V+ percentage.

Figure 2 shows the application of the Moxi Flow to the measurement (PS expression using PE-Annexin V) of the time-course of apoptosis induction with the drug camptothecin. The red curve shows the classic activation curve of capmtothecin-induced apoptosis induction, readily fit with a Hill Equation to extract the activation kinetics (base=5.6%, max=85%, rate=9%/hr, half time=5.7hr). The negative control (blue) shows the expected slight, linear drift in apoptosis expected for cells maintained in experiment culture conditions. Separately, at each time point, measurements of sample viability were taken for each sample (Fig. 3 inset), confirming that both samples exhibited viabilities > 97% throughout the course of the experiment, assuring a minimal and consistent contribution of necrotic cells to the overall Annexin V+ population.

CONCLUSIONS

The data in this technical note show the versatility of the Moxi Flow in monitoring and quantifying apoptosis in both the PE-Annexin V assay and SR-FLICA approaches. The Moxi Flow distinguishes itself from traditional flow systems with its portability, low cost, and minimal training requirements and is uniquely suited for immediate and accurate assays on demand. These features should establish the Moxi Flow as a staple in any lab performing routine apoptosis and viability analysis.

REFERENCES

1. *Favaloro, B., Allocati, N., Graziano, V., De Ilio, C., and De Laurenzi, V. {Role of Apoptosis in Disease. Aging". May, 2012. v4(5), 330–349.*
2. *Kamogashira T., Fujimoto C., and Yamasoba T. "Reactive Oxygen Species, Apoptosis, and Mitochondrial Dysfunction in Hearing Loss". BioMed Research International. 2015. Article ID 617207.*
3. *Kang, P.M. and Izumo, S. "Apoptosis and Heart Failure — A Critical Review of the Literature". Circulation Research. 2000. v86, 1107–1113.*
4. *Lowe, S.W. and Lin, A.W. "Apoptosis in Cancer". Carcinogenesis. 2000. v21(3), 485–495.*
5. *Mattson, M.P. "Apoptosis in Neurodegenerative Disorders". Nature. Nov, 2000. v1, 120–129.*

Description	Cat. No.
Moxi Flow™ Next Gen Flow Cytometer with Pack of u-Flow Cassettes (Type S) and Power Cord	10205-038
Moxi Cyte Flow Reagent (15 mL, 750 tests)	10204-986
Moxi Cyte Viability Reagent (15 mL, 100 tests)	10204-958
Moxi Flow™ Cassette Pack, Type MF-M, Pack of 25	10204-956
Moxi Flow™ Cassette Pack, Type MF-S, Pack of 25	10204-984
Moxi Flow™ System Check Beads (5 mL)	10205-036

Not all ordering options available in Canada.

INTERFERin®:
The Latest Generation siRNA & miRNA Transfection Reagent

The natural RNA interference (RNAi) has become a powerful tool for gene function analysis, gene mapping, or signaling pathway studies. Chemically synthesized small interfering RNA or micro RNA molecules (siRNA or miRNA) are delivered to the cells by transient transfection to modulate expression of exogenous and endogenous genes in mammalian cells at the mRNA and the protein level.

Polyplus-transfection has developed a novel transfection reagent, INTERFERin, that leads to high transfection efficiency in a wide variety of cells to reach great gene silencing (with siRNA or miRNA) or stimulation of gene expression (with miRNA). In addition, INTERFERin reduces off-target effect by achieving efficient and selective gene silencing using only 1nM siRNA for various target genes with no toxicity observed. With its fast and simple protocol, INTERFERin becomes the reagent of choice for siRNA or miRNA transfections.

Reduce Off-target Effect by Using as Little as 1nM siRNA

Efficient gene silencing without undesired off-target effects is of crucial importance to generate accurate and reliable data. Several publications emphasized the importance of using low siRNA concentrations to achieve such results[1,2]. This is why INTERFERin has been specifically designed to provide high silencing efficiency using low siRNA amounts.

INTERFERin-mediated delivery of specific siRNA at 1nM concentration shows selective and highly efficient knockdown of gene expression, while a competitor needs at least 10nM siRNA to reach 50% silencing efficiency (Fig 1A). Cells transfected with 1nM siRNA show no detectable target gene expression while the mismatch siRNA does not lead to any silencing (Fig 1B).

Obtain Over 90% Gene Silencing in a Wide Variety of Cells

Transfection efficiency and gene silencing may vary with cell type and target gene. Thanks to the powerful delivery and low toxicity, INTERFERin achieves efficient and selective gene silencing with 1–5nM siRNA for various target genes in a wide range of adherent, primary and suspension cells (Table 1).

Achieve Excellent Results with miRNA Transfections

INTERFERin is perfectly adapted for delivering miRNA or miRNA-related molecules (pre-miRNA, pri-miRNA, miRNA mimics, or miRNA inhibitors) and leads to high gene silencing or increase in expression of desired gene. Indeed, some miRNA are known to induce gene expression by association with the promoter of the gene of interest. For example, INTERFERin-mediated delivery of miR-373 leads to a 3-fold increase in E-Cadherin expression (Fig 2).

Figure 1A.

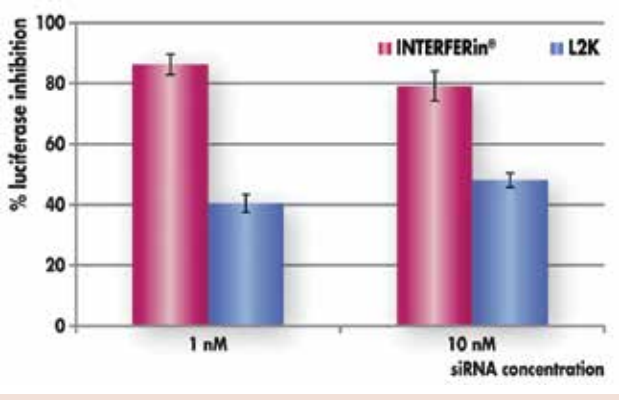


Figure 1B.

Figure 1. INTERFERin® only requires 1nM siRNA for efficient gene silencing A. Inhibition of luciferase expression in 3LL cells stably expressing Firefly Luciferase 48h after transfection with an anti-Luc siRNA using INTERFERin or competitor L2K using manufacturer's recommendations. **B.** CaSki cells were transfected with 1nM lamin A/C or a mismatch siRNA using INTERFERin. After 48h, lamin A/C silencing efficiency was determined by immunofluorescence microscopy.

Polyplus transfection™

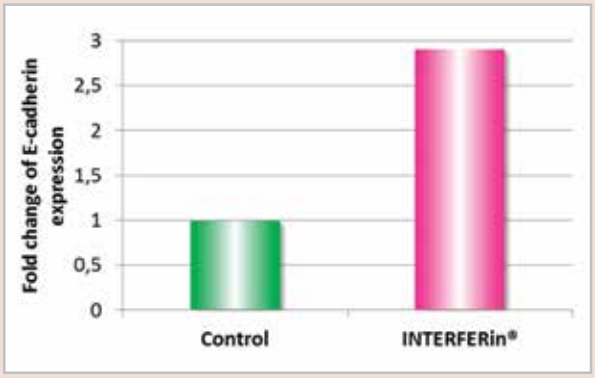


Figure 2. Transfection of miR-373 with INTERFERin enhances E-cadherin expression. PC-3 cells were transfected with 25 nM miR-373.72h after transfection, E-cadherin mRNA expression level was determined by RT-qPCR. The assay was normalizedwith the HPRT-1 gene expression.

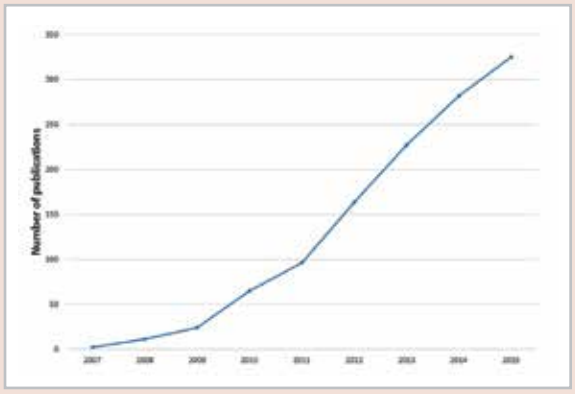


Figure 3. INTERFERin is involved in an increasing number of publications about miRNA transfection (Google Scholar).

miRNAs are increasingly being used to analyze specific biological effects or cell functions. High transfection efficiency associated with high cell viability makes INTERFERin the reagent of choice for generating relevant data for scientific publications. Indeed, the number of publications using INTERFERin for miRNA and miRNA related molecules has been exponentially growing since its launch (Fig 3).

Adherent cell lines (1 nM siRNA)		
A549	Luciferase	> 90%
HeLa	GAPDH / Lamin A/C	
CaSki	GAPDH / Lamin A/C	
MCF7	GAPDH / Lamin A/C	
NIH-3T3	Vimentin	
RAW	Eg5	
SiHa	GAPDH / Lamin A/C	
HepG2	GAPDH	60-70%
Primary cells (1 nM siRNA)		
MEF Murine embryonic fibroblasts	GAPDH	> 90%
Primary human fibroblasts	GAPDH / Lamin A/C	
Primary human hepatocytes	GAPDH	
Suspension cell lines (5 nM siRNA)		
K562	GAPDH	> 80%
THP-1	GAPDH	

Table 1: Some of the cell lines and primary cells successfully transfected with INTERFERin and silencing efficiencies obtained. For adherent cell lines or primary cells, 1nM siRNA is sufficient to obtain more than 90% gene silencing. For suspension cell lines, 80% silencing can still be reached by INTERFERin using 5nM siRNA.

Gentle Mode of Action for More Robust Data and Excellent Cell Viability

When it comes to cell viability, INTERFERin outperforms other transfection reagents. 48h after transfection with 1nM siRNA, cells transfected with INTERFERin appear healthy, while toxicity is clearly observed with competitor reagent S (Fig. 4).

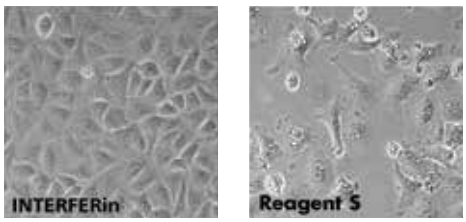


Figure 4. INTERFERin is extremely gentle on cells. Cell morphology of A-549 GL3Luc cells 48h after transfection with 1nM Luciferase siRNA and INTERFERin or competitor reagent S using the manufacturer's protocol.

Easy to Use: Simple Protocol, Compatible with Serum and Antibiotics

INTERFERin is provided as ready-to-use solution with a straightforward, simple protocol. INTERFERin is compatible with both serum and antibiotics, hence forget time consuming medium changes and washes; INTERFERin can be left on the cells without any adverse effects.

References:

1. Birmingham, A. et al., (2006) Nature Methods, Vol.3, No3, 199-204.
2. Caffrey, D. et al., (2011) PLoS One. 2011;6(7):e21503. Epub 2011 Jul 5.

INTERFERin® siRNA Transfection Reagent	Cat. No.
0.1 mL	**89129-928**
1 mL	**89129-930**
5 x 1 mL	**89129-932**

Advantage of CELLSTAR® Cell Culture Vessels with Cell-Repellent Surface for 3-D Cell Culture in Hydrogels

greiner bio-one

Research with two-dimensional (2-D) cell culture, where cells attach to the surface of a cell culture vessel, can mimic only to a limited extent the conditions in physiological tissue, where cells are able to interact in a three-dimensional network. Therefore, results generated from 2-D cultures often have limited relevance for studying cell behavior and function.

An alternative approach to reflect in-vivo conditions more closely is the cultivation of cells in three-dimensional (3-D) systems. One option to mimic a 3-D environment is the usage of hydrogels consisting of chemically defined, synthetic components. Cells cultivated in hydrogels are a valuable source for biochemical analysis like gene expression or metabolic assays of whole 3-D cell populations.

Nevertheless, when long-term incubations of hydrogel-cultures are done in standard tissue culture vessels, some cells tend to migrate out of the hydrogel onto the vessel surface, forming a 2-D subculture (Fig. 1A).

Analysis of such cell populations will therefore result in mixed data from both 2-D and 3-D cell cultures. If CELLSTAR cell culture vessels with cell-repellent surface are used for hydrogel culture, the formation of a 2-D subculture is suppressed effectively (Fig. 1B). The CELLSTAR cell-repellent surface from Greiner Bio-One is achieved through an innovative chemical surface modification and is available with different formats.

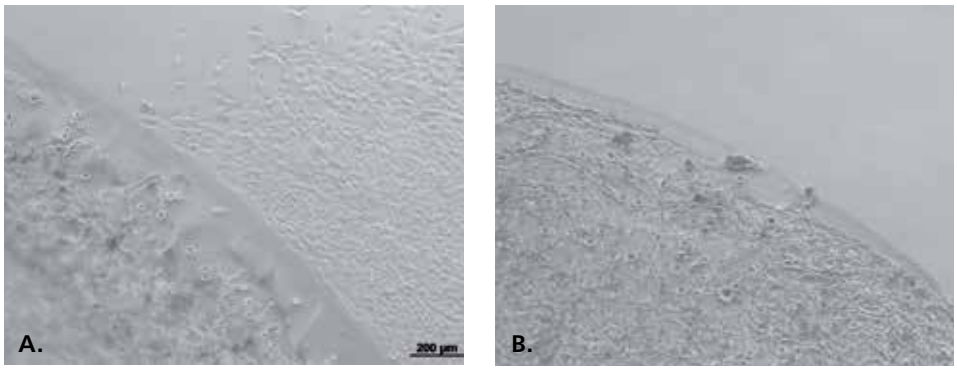


Figure 1. Cell culture vessels with a cell-repellent surface prevent 2-D growth of cells escaping a 3-D hydrogel culture. 3T3 fibroblasts were cultured in 30µL 3-D Life PVA Hydrogel modified with the adhesion peptide RGD (3-D Life RGD Peptide) and cross-linked with a cell-degradable peptide (3-D Life CD-Link) to allow for migration of cells within the gel. Gels were applied to 6-well multiwell plates with a tissue culture (A) or cell-repellent (B) surface and incubated at 37°C in a 5% CO_2 environment over 8 days. Cultures were analyzed by phase contrast microscopy. Experiments were done at Cellendes GmbH, Reutlingen, Germany.

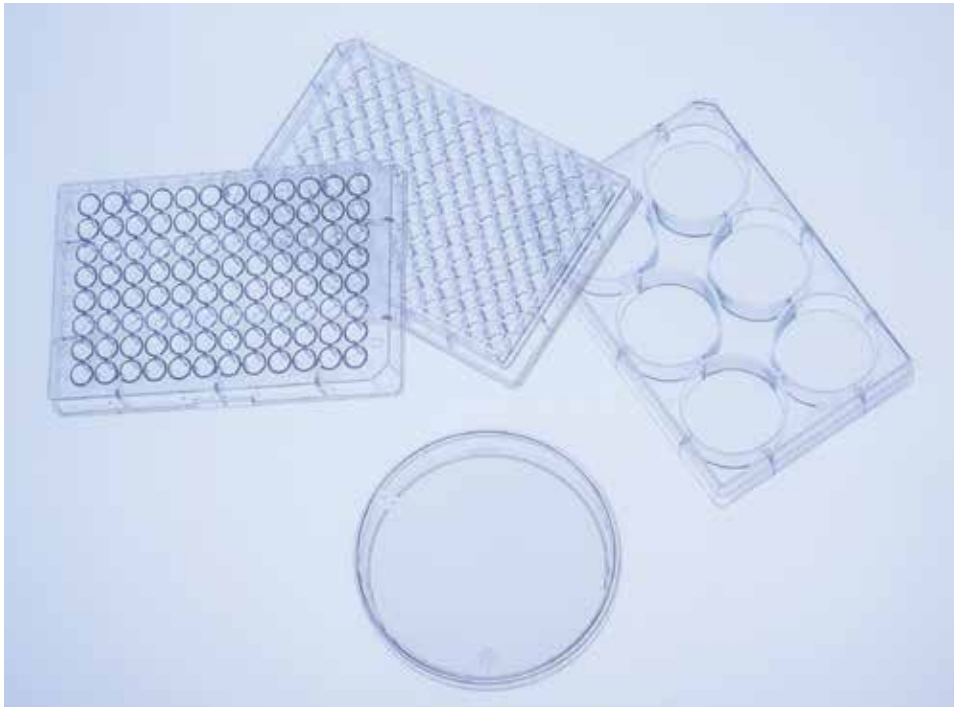


Figure 2. Greiner Bio-One products with CELLSTAR cell-repellent surface

Product Descriptions	Cat. No.
96-Well Cell-Repellent Plate, PS, Sterile, Round (U) Bottom, Clear, w/Lid	30618-026
96-Well Cell-Repellent Plate, PS, Sterile, Flat Bottom, Chimney Style, Clear, w/Lid	30618-028
48-Well CELLSTAR® Cell-Repellent Surface, Sterile, w/Lid	30618-024
24-Well CELLSTAR® Cell-Repellent Surface, Sterile, w/Lid	30618-022

Lonza HL-1™ Supplement

The Chemically Defined and Regulatory Friendly Serum Substitute

Lonza currently offers the HL-1 supplement which is a synthetic alternative to serum that can be used in conjunction with many classical media formulations including: IMDM, RPMI, DMEM, or DMEM:F12. The HL-1 supplement can be used to replace serum altogether or can be used in conjunction with low serum formulations depending on the application.

Due to the supply issues and high cost of serum coupled with lot qualification and lot variability, many researchers are looking for a regulatory friendly product to advance their research. HL-1 supplement is chemically defined and registered via a masterfile with the FDA. The HL-1 supplement provides a solution to customers who want to move away from using fetal bovine serum by providing the necessary proteins and growth factors that are found in serum and needed by most cells.

Many comparison studies have been done with various hybridoma cell lines to show the efficacy of using the HL-1 supplement in place of 5% fetal bovine serum.

Lonza's HL-1 supplement contains less than 30µg/mL protein and has been proven to support the growth of more than 40 different cell types across various species including lymphoma, cancer, and even primary cells.

Little to no adaptation is required when switching from a variety of different media formulations with various concentrations of serum.

Traditionally, serum substitutes are mainly used by stem cell researchers for specific applications, however Lonza's HL-1 supplement offers a flexible serum substitute that can be used with a variety of classical media formulations, cell types, and species to provide a variety of benefits to researchers regardless of application.

Description	Cat. No.
HL-1 Complete Serum-Free Medium without L-Glutamine, 1X	12001-772

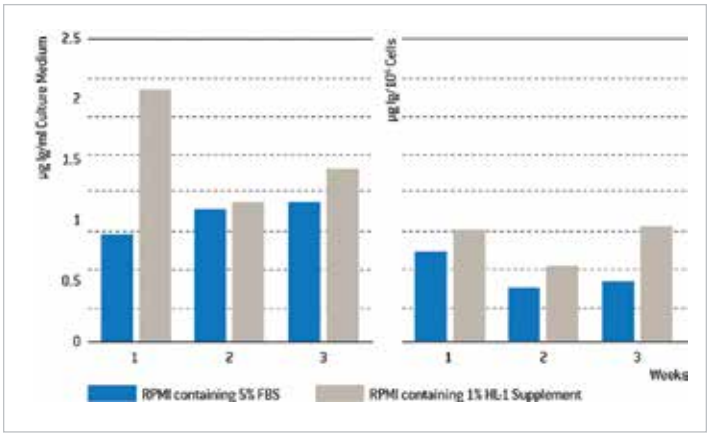


Figure 1: Adaption of a hybridoma cell line to medium containing HL-1 supplement compared to fetal bovine serum. A hybridoma cell line was grown in two different basal media supplemented with 2mM L-glutamine and 15mM HEPES. One media contained 1% HL-1 supplement, while the other media contained 5% fetal bovine serum. The cells were maintained in each media formulation for two weeks and were passaged three times per week. Cells were harvested via a kinetics study. Week 1 and 2 results for HL-1 use are listed as well as week 2 results of media supplemented with fetal bovine serum.

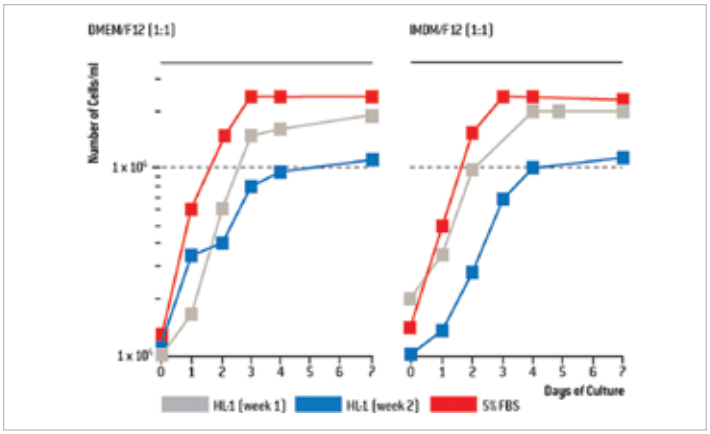


Figure 2: Comparison between monoclonal antibody production by hybridoma cells grown in RPMI-1640 containing either fetal bovine serum or the HL-1 supplement. A hybridoma cell line was cultured in RPMI-1640 for seven days. Cells were maintained in medium containing either HL-1 supplement or 5% fetal bovine serum for three consecutive weeks and were passaged three times per week. The blue bars represent the Ig concentration in the culture which were supplemented with 5% FBS. The gray bars represent the culture which contained 1% HL-1 supplement.

Quantitating Autophagy in Neuronal Cells

CYTO-ID® Autophagy Detection Kit

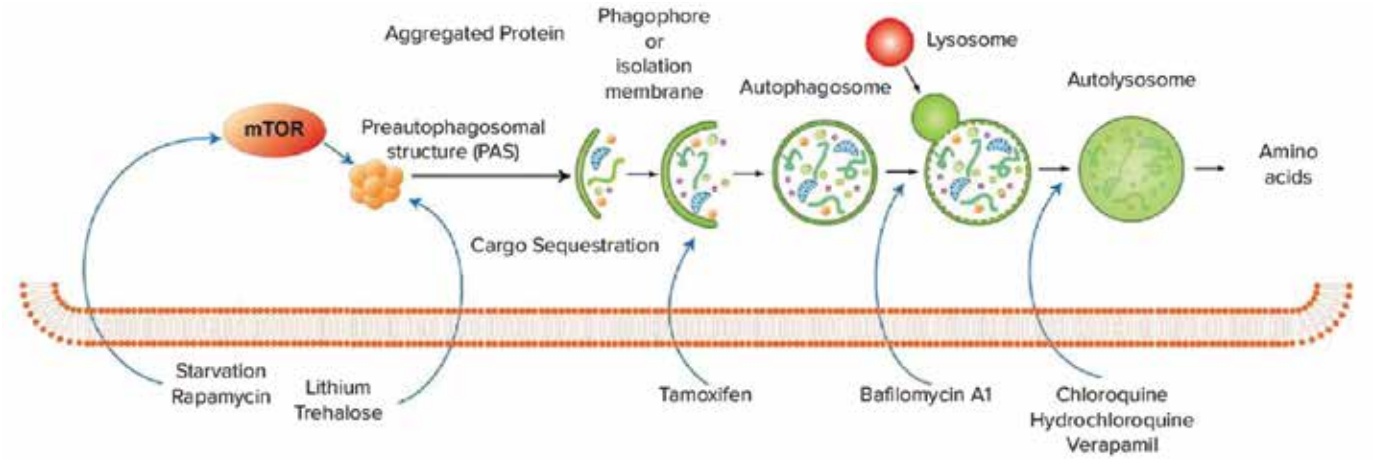


Figure 1: The process of autophagy begins when an internal or external signal promotes the formation of a phagophore, a spherical double-membrane sequestering structure. Microtubule-associated protein 1 light chain 3 alpha (LC3) stimulates elongation of the phagophore, which begins to engulf cytoplasmic targets. In a process mediated by autophagy-related (ATG) proteins, the phagophore closes around the targets to become an autophagosome. The autophagosome then fuses with a lysosome, exposing its contents and its own membrane to degradation by hydrolytic enzymes[2].

OVERVIEW OF THE BIOLOGY

Autophagy is an intracellular catabolic process that sequesters and degrades proteins and organelles that have either been recognized as faulty or are simply no longer needed by the cell. Autophagy supports normal cellular processes by carrying out the "housekeeping" function of removing invasive microorganisms, misfolded proteins, and degraded organelles. Autophagy is essential to the maintenance of cell homeostasis and, in addition to its role as a mechanism of cell material turnover, Autophagy supports the response of an organism to challenges such as starvation by adjusting rates of energy consumption and material re-utilization.

Both beneficial and harmful roles for autophagy have been discovered in cancer, infectious disease, diabetes, neurodegenerative disease, and other conditions. The role of autophagy is of particular interest in the study of neuronal cells, where it helps to maintain protein structure and function over long axonal distances. Unlike the cells of other tissues, the cytosolic contents of neurons are not routinely diluted by mitosis. Altered autophagy and accumulation of cellular toxins are seen in Alzheimer's disease, Parkinson's disease, and other neurological disorders[1]. These observations have led to the consideration of the various mechanisms of autophagy as potential areas of therapeutic interventions. Quantitation of the effects of experimental variables on this process is an essential element of research that may lead to the development of safe and effective treatments targeting autophagy.

VISUALIZING AUTOPHAGY

Autophagy can be visualized and characterized even in heterogeneous cell populations using readily available reagents. CYTO-ID autophagy reagent selectively stains the membranes of phagophores, autophagosomes, and autolysosomes. It has also been shown to co-localize with the autophagosome protein LC3-II. It has a fluorescence excitation/emission profile of 495/519 and can be used with a standard FITC filter set.

QUANTITATION METHODS

The level of CYTO-ID Green staining of autophagosomes in live cells provides information about the mechanism of a compound's stimulation or inhibition of the autophagy pathway. This wasaccomplished using ImageXpress® Micro XLS System (Molecular Devices), a high content screening system for widefield fluorescence or brightfield imaging of fixed or live cells, tissues, and small organisms. Measured values can be plotted over time or against experimental conditions such as a range of drug compound concentrations.

INCREASED NUMBER OF AUTOPHAGOSOMES INDICATES CELL DISTRESS

In the examples that follow, iCell (Cellular Dynamics Intl.) human-induced pluripotent stem cell (iPSC) derived neurons and rat PC-12 cells were used to demonstrate the utility of testing for multiple cellular responses in each well of a 384-well microplate.

Human Neurons

Neurons were plated at 5000 cells/well and grown following the manufacturer's recommendations. After 5 days, cells were treated with a dilution series of compounds prepared in maintenance media. After 24 hours of compound incubation, staining for nuclei and autophagosomes was performed and live cells were imaged using ImageXpress Micro XLS Widefield Automated System with the chamber heated to 37°C. Images were acquired from 2-4 sites per well using either a 40X Plan Apo or 60X Plan Fluor objective. Image analysis was accomplished using MetaXpress software to quantitate the effects of exposure to experimental compounds over a range of concentrations.

Rat PC-12 Cells

PC-12 cells were plated at 2000 cells/well and cultured overnight before treatment with a dilution series of compounds prepared in complete media. After 24 hours, staining for nuclei and autophagosomes was performed and live cells were imaged at 37°C. Images were acquired from 2-4 sites per well at 40X magnification. Image analysis was accomplished using MetaXpress software to quantitate the effects of exposure to experimental compounds over a range of concentrations.

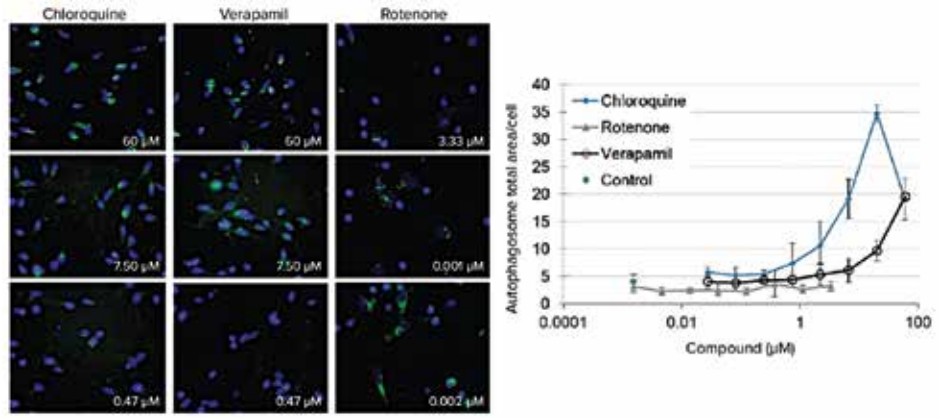


Figure 2. (Left) Representative 60X magnification images of human neurons following exposure to three different compounds at three different concentrations. Autophagosomes (green) respond to the cell treatment in a dose-dependent manner. **(Right)** Dose-response effect of chloroquine, rotenone, and verapamil on aggregate autophagosome area in human neurons. Autophagosomes may be inhibited or stimulated by compound exposure.

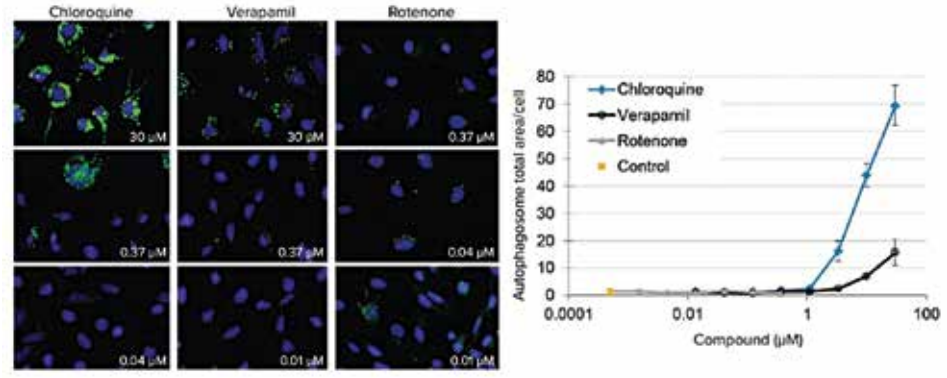


Figure 3. (Left) Representative 40X magnification images of rat PC-12 cells following exposure to three different compounds at three different concentrations. Autophagosomes (green) can be clearly visualized and measured. **(Right)** Dose-response effect of chloroquine, rotenone, and verapamil on aggregate autophagosome area in rat PC-12 cells. Autophagosomes may be inhibited or stimulated by compound exposure.

Summary

Scientific interest in the quantitation of neuronal autophagy is driven, in part, by the potential value of the cellular process of autophagy as a target for therapeutic intervention against neurodegenerative diseases, such as Parkinson's, Alzheimer's, and Huntington's disease. The CYTO-ID Autophagy Detection Kit contains a no-transfection specific dye for fluorescence microscopy visualization of autophagosomes. The ability to assess autophagy in specific neuronal cell cultures relative to experimental conditions offers researchers the opportunity to examine dose-response effects of experimental compounds on neuronal autophagy or to screen libraries of compounds that may affect autophagy and therefore neuronal physiological mechanisms relevant to neurodegenerative diseases[1].

References

1. Jin H. Son, et. al. (2012) Neuronal autophagy and neurodegenerative diseases. Experimental and Molecular Medicine 44, 2:89-98.
2. Mizushima, Yoshimori and Levine (2010) Methods in Mammalian Autophagy Research. Cell 140:313-326.

Description	Cat. No.
Cyto-ID Autophagy Detection Kit	89165-926
Cyto-ID Autophagy Green Long-Term Cell Tracer Kit	10136-012

Not all ordering options available in Canada.

The Unique Design of Thermo Scientific Nunc Carrier Plate Enables Precise Adjustment of Height for Cell Culture Inserts in Multiwell Dishes

Robert Scott, Jim Schantz, Joe Granchelli, and Cindy Neeley

ABSTRACT

The Thermo Scientific™ Cell Culture Insert can be seated in a multiwell dish via either the 1mm feet at the bottom of the insert or the three hanging tabs on the side of the insert. The Thermo Scientific carrier plate with the hanging slots allows for adjustment of insert height by engaging different hanging tabs on the insert. Here we determine, through physical measurement of the complete product system, the height of the growth surface of cell culture inserts when hanging in carrier plates. Measurements were taken for all three hanging positions, and recommended growth media volumes were calculated. Inserts were determined to hang with approximately 0.9mm, 3.3mm, and 6.3mm between the well-bottom and insert growth surface for both 12- and 24-well plate formats.

INTRODUCTION

To facilitate versatility in the usage of cell culture inserts, carrier plates are designed to hold cell culture inserts in multiple positions above the growth surface of the multi-welled dish containing the insert. An important consideration for cell culture researchers is the height of the growth surface of the insert within the well, and the resulting volume of media required to grow cells at each height. Media height and volume are variables which may need to be controlled to optimize such things as gas exchange, oxygen tension, availability of growth factors, or pH. Adjustment of the insert allows more than one physical position relative to the bottom of the carrier plate. Each position has minimum media volume requirements to cover the cells. Physical measurements were made of inserts and carriers and those measurements, as well as recommended media volumes are reported below.

MATERIALS EXAMINED

- 12-well carrier plate/multiwell plate, 8.0µm pore cell culture insert

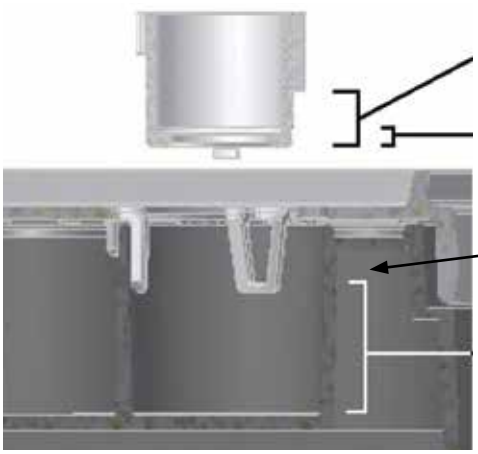
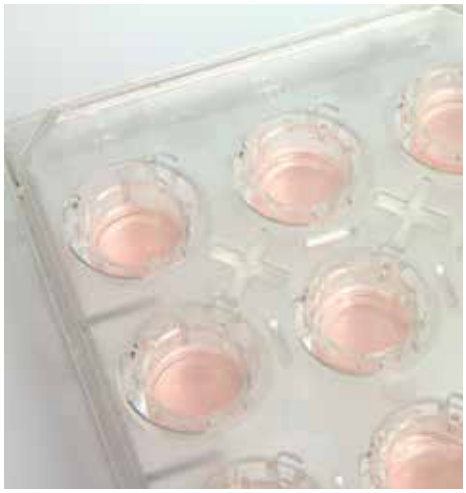


Figure 1. Depiction of measurements taken from cell culture inserts and carrier plate/multiwell dishes.

Height of the cell culture insert growth surface

$$H = C - (A - B)$$



- 24-well carrier plate/multiwell plate, 0.4µm pore cell culture insert

METHODS

To first determine the hanging height in the carrier plate, the distance from bottom surface of the insert to the bottom of each height-adjusting tab on the insert was measured using a height gauge and stationary plate (See Figure 1, A). All three tabs on each insert were measured, and for each carrier plate format (12- and 24-well) a sample of n=3 inserts was included. Since the top of the polycarbonate film is the growth surface, the thickness of the film must be accounted for in measuring height (Figure 1, B). This was measured using a thickness gauge, and the film thickness was subtracted from measurement A to determine the distance from the bottom of the height-adjusting tab to the insert growth surface.

When placed in the carrier plate, the bottom of the height-adjusting tab rests on the hanging slot of the carrier plate.

Thermo SCIENTIFIC

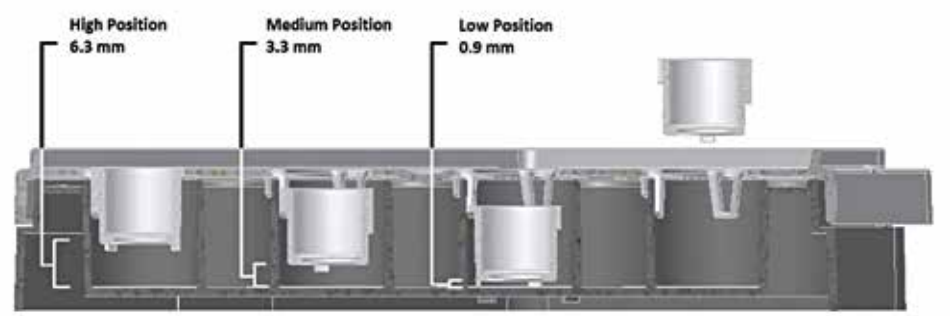


Figure 2. Depiction of cell culture inserts hanging in carrier plate, showing three different hanging positions. Dimensions given represent the height of the growth surface of the cell culture insert from the interior bottom of the well (Height H). Dimensions in figure are rounded to the tenth of a millimeter.

Hanging Position	Measurements	24-well Carrier Plate	12-well Carrier Plate
Low	Height (mm)	0.9 ±0.1	0.9 ±0.1
	Working Volume (mL)	1.0	2.0
Medium	Height (mm)	3.3 ±0.1	3.4 ±0.1
	Working Volume (mL)	1.5	3.0
High	Height (mm)	6.3 ±0.1	6.4 ±0.1
	Working Volume (mL)	2.0	4.0

Table 1. The height of the cell culture insert growth surface for all three hanging positions with recommended working volumes that allow 5mm medium coverage of the insert.

Therefore, the hanging height of the growth surface (measurement A minus measurement B) is identical to the distance from the bottom of the hanging slot to the insert growth surface. To determine the well height of the insert growth surface from the bottom of the well plate, the height of the hanging slot from the well bottom was measured (Figure 1, C) This was performed using a height gauge and stationary plate. Three different carrier plate/multiwell dish samples were measured for each plate format, and measurements were taken from three locations in six different wells. Measured wells were chosen to sample as much of the plate as possible, at all four corners and two wells near the plate center (well B2 and B3 in 12-well, B3 and C4 in 24-well). Individual well measurements were averaged, and the calculation **H = C - (A - B)** was performed using these means for each height-adjusting tab size. The resulting dimensions (height H, see Figure 2 for clarification) represented the height of the cell culture insert growth surface from the bottom interior of the well in each hanging position. Using height H calculated above, the volume of cell growth media needed to cover the insert growth surface was also calculated. To cover cultures with media 5mm deep, 5mm was added to height H for each hanging position. This height was then multiplied by the growth surface area in individual wells for each plate format. The resulting volume is given as the recommended media volume at each hanging position.

RESULTS AND DISCUSSION

Table 1 contains overall results averaged from all plates and inserts for each format. Surface areas used for volume calculations are 3.5 cm^2 for 12-well plates, and 1.8 cm^2 for 24-well plates. Working volumes are calculated to give 5mm growth media over the growth surface. Media volumes shown are suggestions, different volumes may be necessary depending on the cell type and experiment being performed. Figure 2 shows cell culture inserts in the carrier plate, in all three configurations, with average hanging heights labeled for clarity.

CONCLUSION

Carrier plates are convenient for implementing versatility in the experimental design of cell culture inserts.

The three hanging positions in the carrier plate allow for distance of approximately 0.9mm, 3.3mm, or 6.3mm between the well-bottom and insert growth surface for both 12- and 24-well plate formats. Working volume should be adjusted according to the hanging position to suit the application needs.

Reference

Thermo Scientific Nunc Solutions for Cell Culture and Growth brochure.

Pore Size, µm	Pore Density, pores/cm^2	Culture Area, cm^2	No. of inserts/carrier plate	Cat No.
Nunc Carrier Plate System for 24-well Multi-dish – Polycarbonate Membrane				
-	-	-	0	**89177-080**
0.4	<0.85 x 10^8	0.47	24	**89177-082**
3.0	<1.7 x 10^6	0.47	24	**89177-084**
8.0	<0.85 x 10^5	0.47	24	**89177-086**
Nunc Carrier Plate System for 12-well Multi-dish – Polycarbonate Membrane				
-	-	-	0	**89177-072**
0.4	<0.85 x 10^8	1.13	12	**89177-074**
3.0	<1.7 x 10^6	1.13	12	**89177-076**
8.0	<0.85 x 10^5	1.13	12	**89177-078**

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

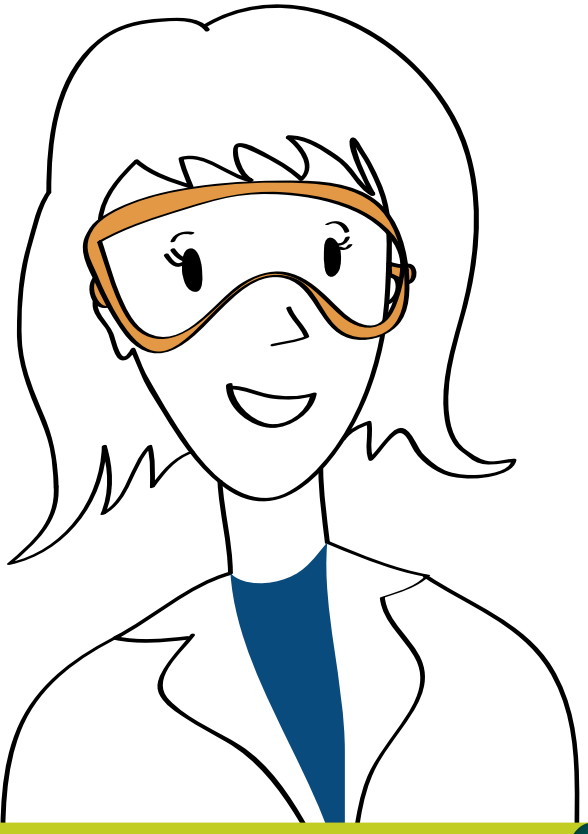
VWR We Enable Science 1.800.932.5000 vwr.com

0915 30M Lit. No. 93533

FSC www.fsc.org MIX Paper from responsible sources FSC® C084269

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

VWR® We Enable Science



Quantitatively and Qualitatively Show Us Your qPCR!

Learn about the qPCR Publication Grant by visiting vwr.com/allyouneedgenomics

Submit your abstract for consideration by January 31, 2016

US ONLY *Not available in Canada.*

VWR® We Enable Science jove Quanta BioSciences, Inc.™