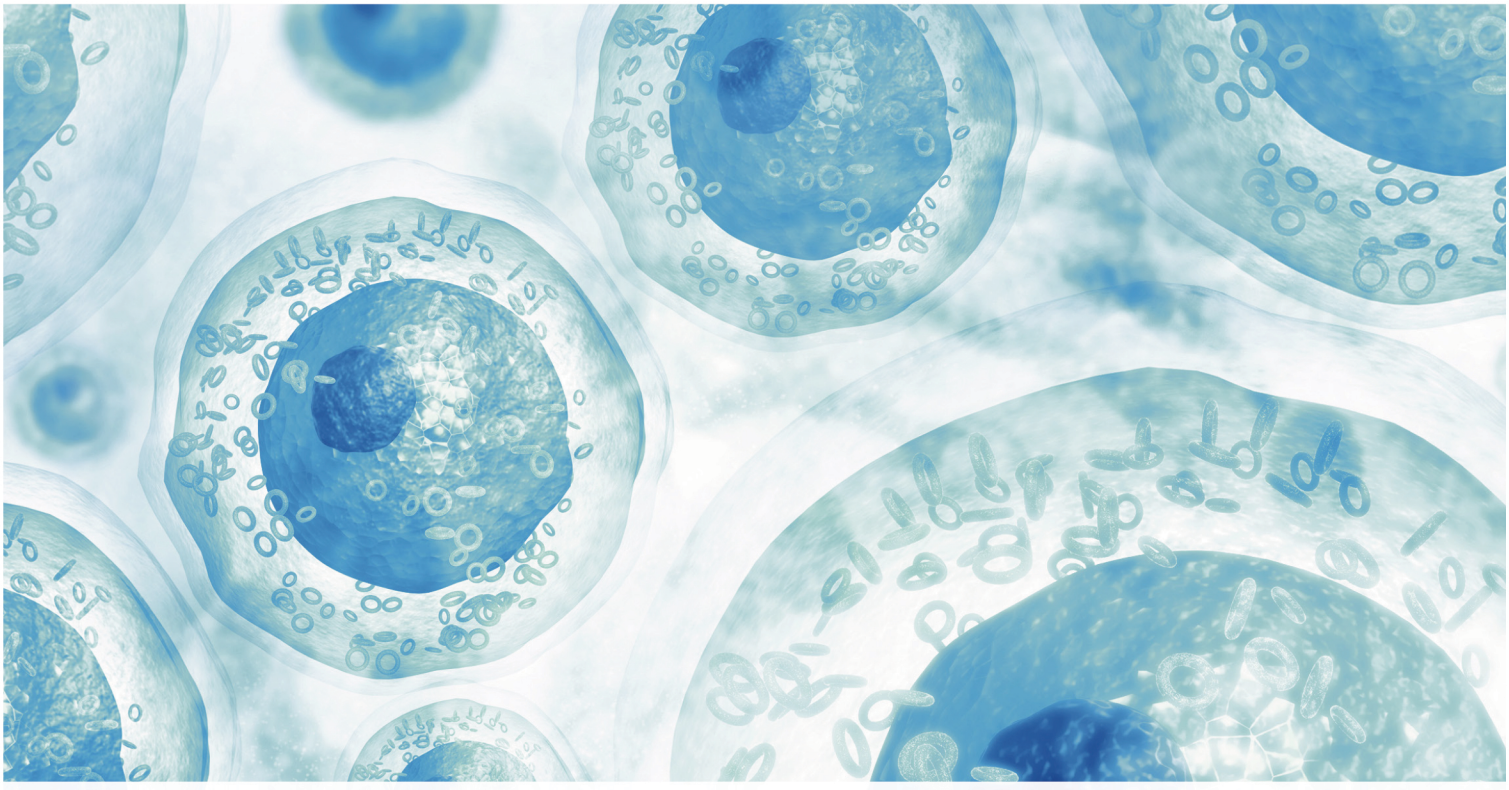
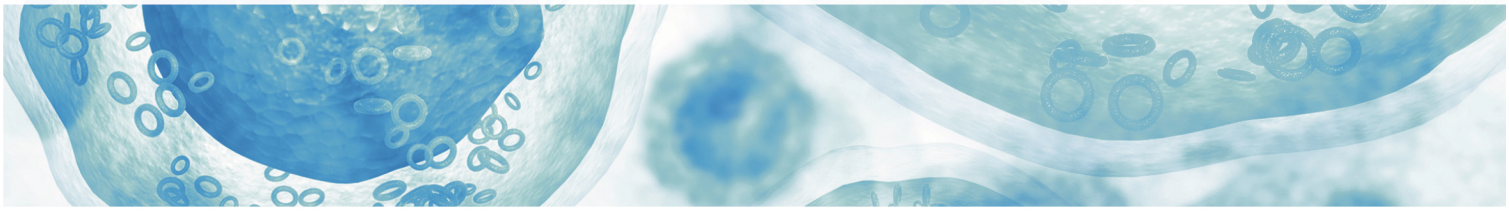


VWR bioMarke

VOLUME 05 ISSUE 01



2019 VWR bioMarke Magazine

A deep understanding of pathological and therapeutic mechanisms, at a cellular and molecular level, is key for the discovery and use of drugs. By providing solutions for your entire workflow, from basic cell culture, gene editing and protein expression research to biopharmaceutical production, we have the new technologies and trusted products to support your efforts to make this world a healthier place.

CELLS TO THERAPY
ISSUE 1

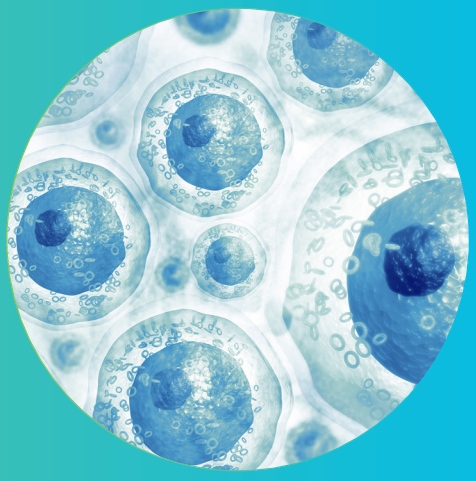


Table of contents

CELLvo™ Human Articular Chondrocytes (HC-A) exhibit native chondrocyte phenotype

By Travis Block PhD, Rachel Leeth, and Anne Sheldrake

Cartilage damage as a result of traumatic injury or long-term wear is the major cause of disability worldwide and a significant health priority for the rapidly aging global population. To better understand mechanisms of cartilage damage and repair, it is desirable that investigators be able to study chondrocytes *in vitro*.

INTRODUCTION

While *in vitro* work with human cells is preferred to non-human cell lines, in order to improve relevance and translational potential of discoveries, major obstacles exist. Sources of human primary tissue for research are severely limited, and chondrocytes rapidly undergo dedifferentiation during *in vitro* expansion. In chondrocytes, dedifferentiation is marked by reduced expression of type II collagen (Col2) and an increase in expression of type I collagen (Col1). Col2 is the structural backbone of healthy cartilage and makes up more than 90% of collagen found in articular cartilage. In contrast, Col1 is present in very small amounts or is absent altogether but, is abundant in fibrocartilage. Together, these factors limit the availability of human chondrocytes possessing a biologically-relevant phenotype.

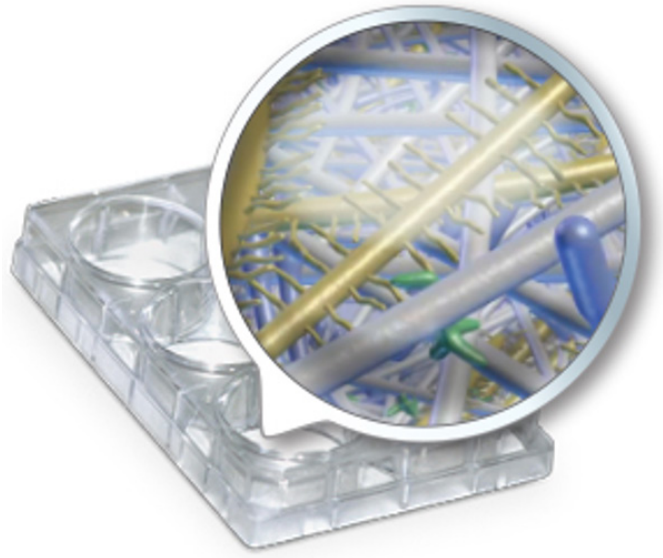
Most commercially available chondrocytes have already been expanded and therefore have already sustained a significant change in their native phenotype. To catalyze progress in this space, StemBioSys® maintains a stock of commercially-available cryopreserved P0 chondrocytes. Furthermore, CELLvo™ Matrix has recently been shown to improve the isolation and expansion of human chondrocytes, while minimizing significant change in phenotype (Mao et al.). Here, we compare gene expression of CELLvo™ Human Articular Chondrocytes (HC-A) to other commercially available articular chondrocyte products. Additionally, we examine the effect of culture on substrate choice (CELLvo™ Chondrocyte-Derived (CD) Matrix (in development) vs. tissue culture plastic (TCP)) on chondrocyte gene expression. We show that CELLvo™ HC-A are the only chondrocytes with a native phenotype, and that CELLvo™ CD Matrix minimizes loss of phenotype during *in vitro* expansion.

These new tools have the potential to facilitate improved basic and translational research in this field.

MATERIALS AND METHODS

Study Design

CELLvo™ HC-A were isolated from fresh articular cartilage and frozen as primary cells. For comparison, competitor cells were purchased from two large cell manufacturers at the lowest passage available (P1 and P2, respectively).



Upon thawing, both CELLvo™ HC-A and competitor cells were prepared for gene expression analysis. Additionally, CELLvo™ HC-A and competitor cells were seeded on StemBioSys' CELLvo™ CD Matrix and TCP for one passage. Proliferation, morphology and phenotype were compared.

Substrate and Media Preparation
CELLvo™ CD Matrix was prepared using protocols adapted from those previously described (Chen et al. 2007). Briefly, HC-A were seeded onto fibronectin-coated plates. After being induced to secrete an extracellular matrix on the surface of the dish, the plates were decellularized using low concentration detergent. The remaining decellularized matrix was washed and then dried for future use.

Cell Culture
Cells were cultured in a MEM supplemented with 15% fetal bovine serum, 1% antibiotic/antimycotic, and 1% GlutaMax™ supplement. Cells were seeded at 10,000 cells/cm^2 and incubated at 37°C with media changes every three days. When cells reached 90% confluence, they were trypsinized and counted using an automated cell counter.

Gene Expression
Gene expression was determined by reverse transcription polymerase chain reaction (RT-PCR). Immediately after thawing vials, approximately 5x10^5 cells per group were rinsed with cold (4°C) phosphate buffered saline and collected in cold (4°C) Trizol. RNA was isolated by phenol-chloroform extraction and 1µg cDNA was synthesized from measured RNA. cDNA was diluted and mixed with TaqMan™ master mix and primers at a 2:3 ratio.

RESULTS

CELLvo™ Primary HC-A exhibit a native phenotype compared to Competitor's (passaged) HC-A
Native chondrocytes are identified based on their localization in cartilage and the expression of a high ratio of Col2 to Col1. The Col2/Col 1 ratio is often used as a surrogate for chondrogenic potential (ability to form cartilage matrix) and as the key marker of "healthy" chondrocytes (Pei and He 2012). To test the "quality" of commercially available chondrocyte products, the Col2/Col1 gene expression was measured immediately after thawing. CELLvo™ primary HC-A exhibited a Col2/Col1 ratio 37 times higher than competitors (Figure 1). Most HC-A on the market today are sold after the first passage. However, after expansion most HC-A have already lost a significant portion of Col2 expression.

CELLvo™ HC-A expanded on CELLvo™ Chondrocyte Matrix retained a better chondrocyte phenotype
To minimize costs and facilitate large studies, especially *in vivo* studies, it may be desirable to expand chondrocytes for one or more passages in order to achieve large cell numbers.

Unfortunately, it is difficult to maintain cells that resemble native chondrocytes using traditional culture tools. Because extracellular matrices contain important cues for regulating cell behavior and phenotype (Marinkovic et al. 2016), we expanded commercially available chondrocytes on CELLvo™ CD Matrix and TCP.

After thawing, only CELLvo™ HC-A had a high Col2/Col1 ratio (Fig 1) and only when cultured on CELLvo™ CD Matrix could those cells

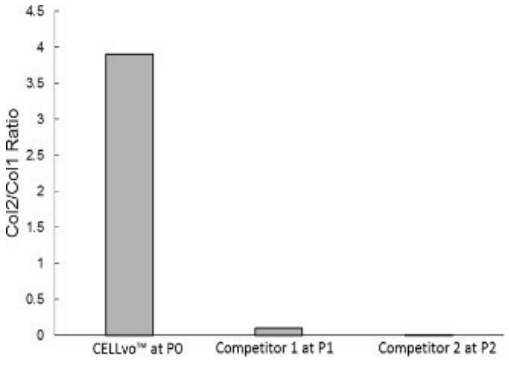


FIGURE 1: **Gene expression measured by RT-PCR immediately after thawing.** Gene expression was determined by the delta-delta Ct method. Expression of Col2 and Col1 was normalized to the housekeeping gene GAPDH.

Cellvo™	PO→P1 E1 (days to confluency)	P1→P2 E2 (days to confluency)	P0→P2 E1*E2	PDLs
Cellvo™ CD Matrix	15 (10)	20.5 (5)	307.5	8.25
TCP	9.25 (10)	18.5 (5)	171.1	7.42
Competitor 1				
Cellvo™ CD Matrix	20.5 (5)	26.1 (4)	535.05	9.04
TCP	7 (10)	17.5 (5)	12.5	6.936
Competitor 2				
Cell™ CD Matrix	14.25 (5)	19.5 (4)	277.9	8.11
TCP	7.25 (10)	21.5 (5)	155.9	7.09

TABLE 1: The fold of expansion (E1) was calculated as the average cell number at first passage divided by the average cell number seeded. The fold of expansion (E2) was calculated as the average cell number at second passage divided by the average cell number seeded. The total folds of expansion from start to finish= E1 x E2. The Population Doubling Level (PDL) was calculated using the formula: PDL=Log10 (N/N0) x3.32 in which N=number of cells at the time of harvesting and N0=number of cells at the seeding time. PDL on each substrate was calculated using the average number of cells at first passage to the last passage.

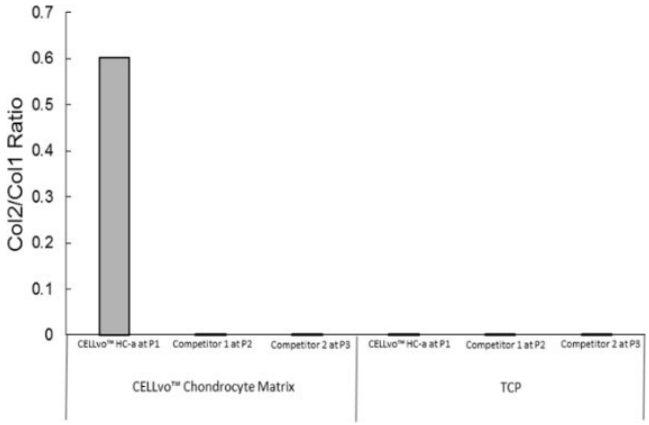


FIGURE 3: **Relative gene expression of cells after culture on CELLvo™ CD Matrix or TCP.** Gene expression was determined by the delta-delta Ct method. Expression of Col2 and Col1 was normalized to the housekeeping gene GAPDH. Preservation of chondrocyte phenotype is often expressed as a ratio of Col2/Col1.

retain elevated Col2/Col1 after expansion. For all HC-A products, culture on CELLvo™ CD Matrix drastically increased proliferation rate (Table 1). However, only CELLvo™ HC-A showed stellate morphology (Fig 2) or decreased dedifferentiation (Fig 3) when cultured on CELLvo™ CD Matrix. The inability to detect phenotype retention in other cell products may be because the initial phenotype already had little resemblance to native chondrocytes.

DISCUSSION

In the present study, CELLvo™ HC-A was the only commercially available human chondrocyte product with gene expression resembling native cells. Moreover, culture on CELLvo™ CD Matrix accelerated proliferation, while minimizing loss of phenotype. Together, these products enable investigators to affordably obtain high quantities of high-quality chondrocytes for research use. In doing so, StemBioSys has eliminated substantial barriers to effective translational research aimed at cartilage repair and regeneration.

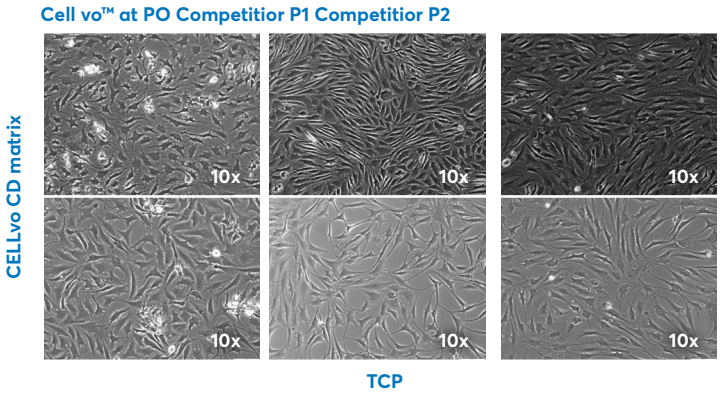


FIGURE 2: **Brightfield images of chondrocytes in culture on CELLvo™CD Matrix vs. TCP.** CELLvo™ HC-A on CELLvo™ CD Matrix (top left), exhibit a stellate morphology typical of chondrocytes. All other groups exhibit a more spindle-like, fibroblast morphology.

REFERENCES

1. Mao Y et al. Extracellular matrix derived from chondrocytes promotes rapid expansion of human primary chondrocytes *in vitro* with reduced dedifferentiation. Acta Biomat, Accepted.
2. Chen XD et al. 2007. Extracellular matrix made by bone marrow cells facilitates expansion of marrow-derived mesenchymal progenitor cells and prevents their differentiation into osteoblasts. JBMR.
3. Pei M, He F. 2012. Extracellular matrix deposited by synovium derived stem cells delays replicative senescent chondrocyte dedifferentiation and enhances redifferentiation. Physiol.
4. Marinkovic M et al. 2016. One size does not fit all: developing a cell- specific niche for *in vitro* study of cell behavior. Bio.

US ONLY

Description	Cat. No.	Unit
CELLvo™ Primary HC-a Cells	76203-918	Each
CELLvo™ Primary HC-a Cells + Sleeve of 5 CELLvo™ XF Matrix 6-Well Plates	76203-920	Each
CELLvo™ Primary HC-a Cells + Sleeve of 5 CELLvo™ XF Matrix T75s Flasks	76203-922	Each
CELLvo™ Primary HC-a Cells + Sleeve of 5 CELLvo™ XF Matrix T150s Flasks	76203-924	Each
CELLvo™ (P1) HC-a Cells	76203-926	Each
CELLvo™ (P1) HC-a + Sleeve of 5 CELLvo™ XF Matrix 6-Well Plates	76203-928	Each
CELLvo™ Primary (P1) HC-a Cells + Sleeve of 5 CELLvo™ XF Matrix T75s Flasks	76203-930	Each
CELLvo™ Primary (P1) HC-a Cells + Sleeve of 5 CELLvo™ XF Matrix T150s Flasks	76203-900	Each

At the time of publication, these products were not available in Canada. Please contact your VWR Life Science Specialist to learn about similar options available in your region.

VWR Life Science cell culture media and reagents

CLASSICAL MEDIA, BUFFERED SALT SOLUTIONS, AND SUPPLEMENTS & REAGENTS

Dulbecco's Modified Eagle's Medium (DMEM)
A modification of Basal Medium Eagle (BME), Dulbecco's Modified Eagle's Medium contains approximately four times as much vitamins and amino acids and two to four times as much glucose as the original formulation. DMEM is suitable for most types of cells, including human, monkey, hamster, rat, and mouse. DMEM is available in a variety of formulations including high or low glucose, with or without sodium pyruvate, and with or without L-glutamine.

RPMI-1640
RPMI-1640 was developed by Moore et al. at Roswell Park Memorial Institute, hence the acronym RPMI. The formulation is based on the RPMI-1630 series of media utilizing a bicarbonate buffering system and alterations in the amounts of amino acids and vitamins. RPMI-1640, when properly supplemented, has demonstrated wide applicability for supporting growth in many types of cultured cells.

Dulbecco's Phosphate Buffered Saline (DPBS)
Dulbecco's Phosphate Buffered Saline is a balanced salt solution used for the handling and culturing of mammalian cells. Phosphate buffering maintains the pH in the physiological range. Calcium and magnesium facilitate cell binding and clumping. DPBS without these ions can be used to wash and rinse suspended cells.

Hank's Balanced Salt Solution (HBSS)
Hank's Balanced Salt Solution is a buffer used to maintain a physiological pH for cells maintained in non-CO_2 atmospheric conditions. HBSS can be used for washing cells or as a solution for transporting cells and tissue culture.

L-glutamine
L-glutamine is an essential amino acid. It is used as an energy source for cells in cell culture. L-glutamine is stable as a dry powder and as a frozen solution but degrades rapidly in liquid media or stock solutions.

Trypsin (0.25%) EDTA
Trypsin (0.25%) EDTA (1X) is used to release adherent cells from tissue culture plasticware for harvesting and passaging. Contains Trypsin=0.25%, EDTA=0.04%.

Description	Size, mL	Cat. No.	Case of
DMEM with 1.0 g/L Glucose, L-Glutamine, and Sodium Pyruvate	500	**VWRL0111-0500**	10
DMEM with 1.0 g/L Glucose, without L-Glutamine, and with Sodium Pyruvate	500	**VWRL0149-0500**	10
DMEM with 4.5 g/L Glucose, L-Glutamine, and Sodium Pyruvate	500	**VWRL0101-0500**	10
DMEM with 4.5 g/L Glucose, L-Glutamine, and without Sodium Pyruvate	500	**VWRL0100-0500**	10
DMEM with 4.5 g/L Glucose, without L-Glutamine, and with Sodium Pyruvate	500	**VWRL0148-0500**	10
DMEM with 4.5 g/L Glucose, without L-Glutamine, and without Sodium Pyruvate	500	**VWRL0102-0500**	10
DPBS 1X, without Calcium Chloride and Magnesium Chloride	100	**VWRL0119-1000**	6
DPBS 1X, without Calcium Chloride and Magnesium Chloride	500	**VWRL0119-0500**	10
HBSS 1X, without Calcium, Magnesium or Phenol Red	500	**VWRL0121-0500**	10
L-Glutamine, 100X, 200mM	100	**VWRL0131-0100**	48
PRMI-1640, with L-Glutamine	500	**VWRL0105-0500**	10
PRMI-1640, without L-Glutamine	500	**VWRL0106-0500**	10
Trypsin-EDTA, 0.25%, 1X	100	**VWRL0154-0100**	48

VWR Life Science Seradigm Fetal Bovine Sera (FBS) and FBS alternatives

Learn more about the fastest growing, global sera brand in the market! **vwr.com/seradigm**

ISIA Traceability Certified Sera

Cell culture experts everywhere are discovering how well their cells thrive on nutritionally superior sera from VWR Life Science Seradigm. Our unique collection and manufacturing techniques preserve more of the naturally-occurring growth factors in sera that cells require to thrive. Our proven track record for performance and consistency enables sera users to switch to a brand that yields more of the results they seek — no matter the application, scale, or budget.

		FBS Products				FBS Alternatives	
		Ultimate Grade FBS	Premium Grade FBS	USDA Approved Origin FBS	Ultra Low IgG FBS	FB Essence	Bovine Calf Serum, Iron Supplemented
Cat. No.*	Unprocessed	**89510-198, 97068-101**	**89510-194, 97068-085**	**89510-182, 89510-186**	**10018-828, 10018-826**	**10805-184, 10799-390**	**10158-360, 10158-358, 10153-134**
	Heat-Inactivated	**89510-200, 97068-107**	**89510-196, 97068-091**	**89510-184, 89510-188**	**10018-832, 10018-830**	**10799-384, 10803-034**	—
Country of Origin	100% US Origin	X	X		X	X	X
	USDA Approved Origin (Non-US)			X			
Product Features	Single-Use Filtration Technology	X	X	X	X	X	X
	Cold Temperature Manufactured	X	X	X		X	
	Class 100 Cleanroom Manufactured	X	X	X		X	
	Triple 0.1µm Sterile Filtered	X	X	X	X	X	
	Whole-lot Homogenous Technology	X	X	X	X	X	X
	Independently Tested by Third-Party Laboratories	X	X	X	X	X	X
Specifications	ISIA† Traceability Certified Sera	X	X	X	X	X	X
	Endotoxin**	≤ 10	≤ 20	≤30	≤ 20	≤ 20	≤ 20
	Hemoglobin**	≤ 25	≤ 25	≤30	≤ 25	≤ 25	≤ 30
	9CFR Virus Testing, Sterility, Mycoplasma	X	X	X	X	X	X
	Biochemical Assay	X	X	X	X	X	X
	Electrophoretic Profile	X	X	X	X	X	X
	Additional Virus Testing by qPCR Technology	X					

* Gamma Irradiation available upon request

** The care and attention devoted to the manufacture of our sera generates final test results that are substantially lower than set specifications. See lot-specific Certificates of Analysis for actual test results.

***International Serum Industry Association (serumindustry.org)

Electroporation optimization:

A user's guide

For more information, visit **vwr.com/mirusbio**

By Anthony Lauer and Laura Juckem, Mirus Bio LLC, Madison, Wisconsin USA

INTRODUCTION

Electroporation is the application of an electric field pulse that results in changes in the cellular membrane, promoting the uptake of exogenous macromolecules including DNA, RNA and proteins. Plasmid DNA is commonly electroporated into mammalian cells to overexpress a gene of interest. Cell and molecular biologists commonly utilize electroporation methods due to its low cost, ease-of-use, and high efficiency delivery of various types of cargo.

The mechanism of electroporation of mammalian cells is poorly understood; however, the most recognized theory suggests that the rapid administration of an electrical pulse creates transient leaky structures or pores in the cellular membrane that allow entry of the nucleic acid or other macromolecules into the cell interior. The DNA, RNA or protein do not require modification or complexing with lipids or polymers which can be beneficial for therapeutic applications. The key experimental factors for mammalian electroporation experiments include: nucleic acid and cell concentration, pulse strength, and buffer composition. Each cell type has specific experimental conditions that will allow for high efficiency electroporation of the macromolecule of interest with minimal cytotoxicity.

Electroporation devices can solicit three types of electrical wave forms including: exponential decay, square wave and time constant (Figure 1). Exponential decay pulses can be optimized by varying the voltage and capacitance settings to achieve a wide pulse gradient. Square wave pulses are characterized by the voltage, time of the pulse, duration between pulses, and the total number of pulses. Time constant pulses specify a single sustained voltage for a designated time. Exponential and square wave are the most common pulses utilized for mammalian electroporation studies.

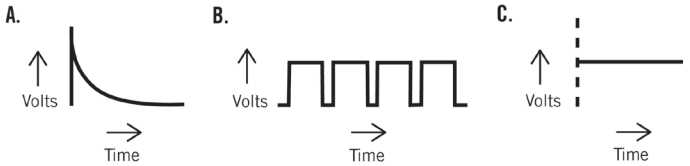


FIGURE 1: **Three types of electrical wave forms.** A shows an exponential decay pattern, B shows a square wave pattern, and C shows a time constant wave pattern.

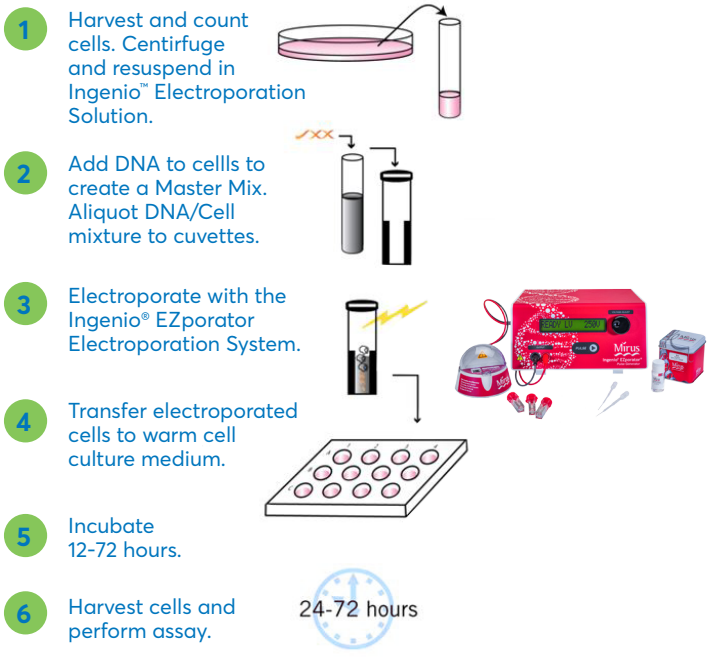


FIGURE 2: **Overview of electroporation workflow**

Specifically, an exponential decay pulse releases the charge from a capacitor set to a specific maximum voltage (Vmax), which then decays exponentially, typically over milliseconds. The delivered pulse is characterized by the field strength (kV/cm) and

the time constant (T) which is the time elapsed before the voltage reaches 1/3 Vmax. The time constant is influenced by both the set capacitance and resistance of the system.

MAMMALIAN CELL ELECTROPORATION PROTOCOL

The process of electroporating mammalian cells is straightforward (Figure 2, page 13). For all conventional electroporators, the cells are harvested from the tissue culture plate, counted, and resuspended at a high density in the electroporation buffer. The recommended cell density depends on the size of the cells: 1 x 10^7 cells per mL for smaller cells such as immune cells or 5 x 10^6 cells/mL for larger cells such as HeLa. The plasmid DNA, or other macromolecule to be delivered to the cells, is added to the cell mixture and transferred to the electroporation cuvette. This contains the electrodes that will conduct the pulse generated from the electroporation device. The cells are then electroporated at a designated voltage and capacitance. Post-electroporation the cells are transferred to a tissue culture plate containing warm complete growth medium. The incubation and harvest time will vary depending on the nature of the experiment. Typically, cells are harvested 24-72 hours post-electroporation and assayed for gene expression or gene regulation.

Pulse Strength

A key parameter to achieve successful permeabilization of the cell membrane is the Field Strength (E) that is applied to the cell. Field strength is calculated using the initial peak voltage that is applied during an exponential decay pulse divided by the gap size of the cuvette (kV/cm). Mammalian cells are less tolerant of high voltage settings than prokaryotes, so a lower field strength must be applied to maintain cell viability. Typically starting voltages range from 80–160 V for a 0.2cm cuvette or 200–300 V for a 0.4cm cuvette.

A titration of the pulse strength was performed in Jurkat E6-1 cells using 0.2cm cuvettes and the Ingenio® Electroporation Solution with the Ingenio® EZporator Electroporation System (Figure 3A). Three

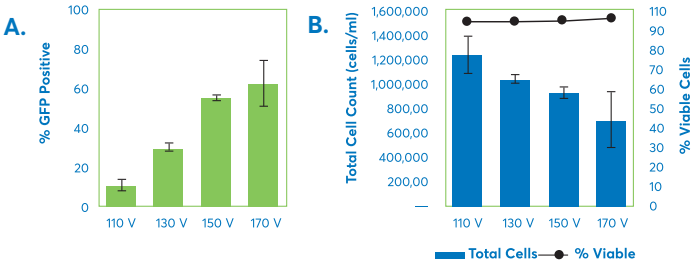


FIGURE 3: Pulse strength affects electroporation efficiency and total cell count. Jurkat E6-1 cells were electroporated with an EGFP reporter plasmid at varying voltages using the Ingenio® Electroporation Solution (Mirus Bio) and the Ingenio® EZporator® Electroporation System (Mirus Bio). GFP efficiency (A) or cell count and viability (B) using propidium iodide was determined by flow cytometry at 48 hours post-electroporation.

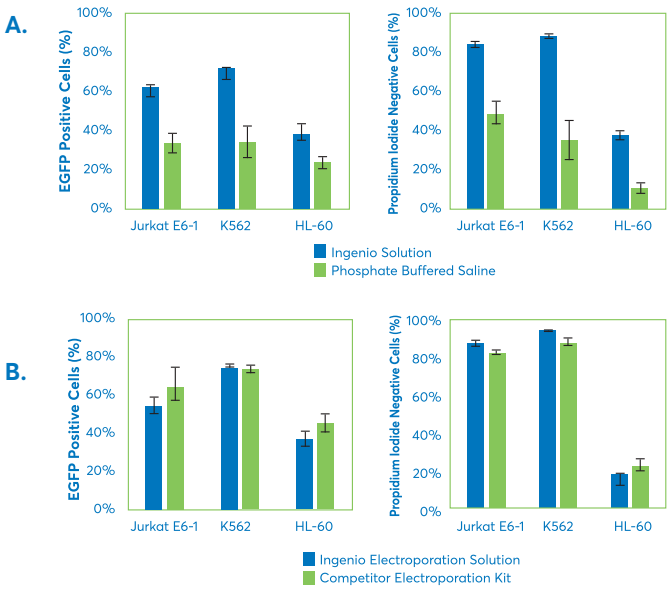


FIGURE 4: Buffer composition influences electroporation efficiency and cell viability. Cells were electroporated in parallel with an EGFP reporter plasmid using either (A) Ingenio® Electroporation Solution or Phosphate Buffered Saline (PBS) on the Gene Pulser Xcell™ Eukaryotic System (Biorad) or (B) Ingenio® Electroporation Solution or competitor electroporation kit using Amaxa® Nucleofector® II Device (Lonza). GFP efficiency and cell viability using propidium iodide was determined by flow cytometry at 24 hours post-electroporation.

parameters were assessed including: (1) the effectiveness of the gene delivery (% GFP positive cells), (2) number of viable cells, and (3) total cell count. The goal in a voltage titration experiment is to determine the voltage conditions that maximizes these three parameters to achieve high transgene expression and to maintain healthy cells.

Electroporation Buffer

The composition of the buffer utilized during the electroporation process is critical to achieving high efficiency and to maintaining low toxicity results. Phosphate buffer saline (PBS) or cell culture medium is frequently used as an electroporation buffer; however, these can have detrimental effects on plasmid DNA delivery efficiency and cell viability (Figure 4A). We observed a lower number of GFP positive cells, and lower cell viability measured by propidium iodide exclusion, when Jurkat E6-1, K562 or HL-60 cells were electroporated in a PBS solution compared to the Ingenio® Electroporation Solution (Mirus). We also tested another commonly used solution in combination with the Amaxa® Nucleofector IIb Device. Similar plasmid DNA efficiencies and cell viabilities were observed between the Ingenio® Electroporation Solution and the competitor solution in Jurkat E6-1, K562 or HL-60 cell types when electroporated using the same program on the Amaxa® Nucleofector® IIb Device (Figure 4B).

Cell Type Specificity

The maximum transfection efficiency that can be obtained for different cell types is based on factors including their size and

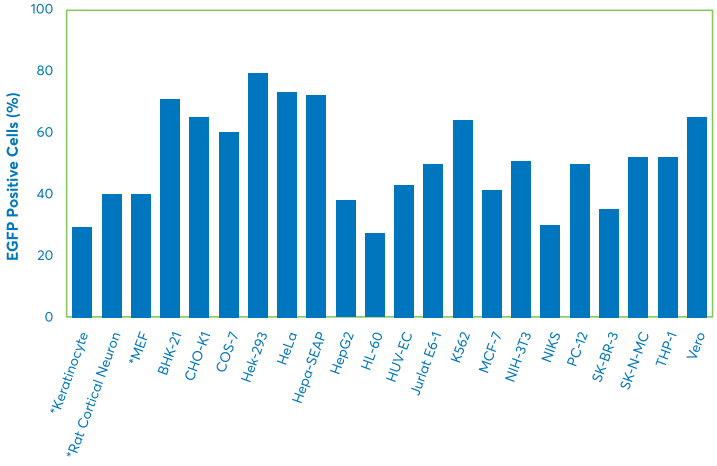
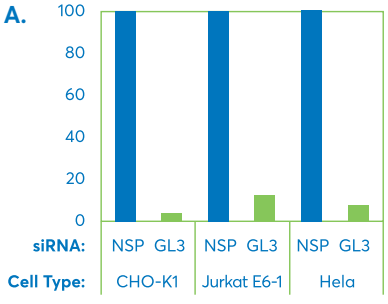


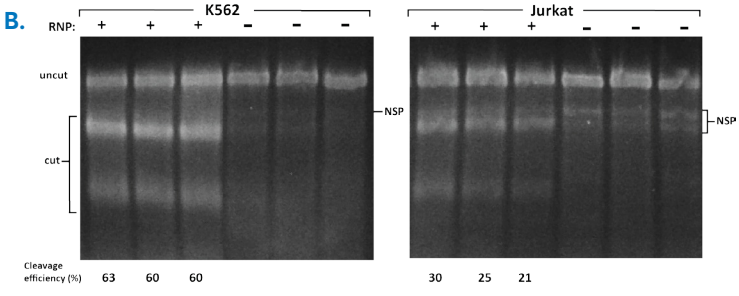
FIGURE 5: Efficient plasmid DNA delivery to a wide variety of cell types. Ingenio® Electroporation Kits were used to transfect indicated cell types using the Amaxa® Nucleofector® IIb Device (Lonza). Cells were assayed at 24 hours by flow cytometry and bars represent the percentage of live cell population expressing GFP. The cell types with an asterisk are primary cells.

membrane composition, so field strength must be selected for each cell accordingly. Optimization of electroporation conditions involves the manipulation of pulse parameters such as: voltage, capacitance and resistance. Application of the optimal field strength allows for the most effective electropermeabilization of the membrane, and thereby delivery of nucleic acid and/or protein. Figure 5 demonstrates representative electroporation efficiencies using optimized pulses to deliver plasmid DNA in twenty-two common and hard-to-transfect cell types including primary keratinocytes, rat cortical neurons and mouse embryonic fibroblasts (MEF).



DELIVERY OF DIFFERENT MACROMOLECULES

While plasmid DNA is the most common macromolecule, the same electroporation conditions can also be utilized to deliver macromolecules of other sizes.



siRNA

RNA interference (RNAi) is mediated by small (21-23 base pair) double stranded RNA molecules that interact with cellular proteins and mRNA in the cytoplasm. A luciferase system can be utilized to determine the quantitative levels of RNAi knockdown of an exogenous gene.

Three cell lines (CHO-K1, Jurkat E6-1, or Hela) were co-electroporated with plasmid DNA encoding GL3 luciferase and either a non-targeting siRNA control or a GL3 targeting siRNA using the Ingenio® Electroporation Solution. Luciferase expression was measured 24 hours post-electroporation and knockdown was calculated by normalizing expression levels to the non-targeting control (Figure 6A). Greater than 80% knockdown of luciferase was observed in all three cell lines.

CRISPR-Cas9

Genome editing using CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) involves the delivery of an endonuclease, Cas9, and a gRNA specific to the genomic DNA site for cleavage. Electroporation can effectively deliver Cas9 and gRNA in various forms, including ribonucleoprotein (RNP) complex comprised of an intact Cas9 protein and a synthetic gRNA.

Electroporation of an RNP complex was performed to target the WT1 Associated Protein (WTAP) locus in K562 or Jurkat E6-1 cells using the Ingenio® Electroporation Solution (Figure 6B). Triplicate 0.2cm cuvettes demonstrate high cleavage at the WTAP locus in both immune cell lines; CRISPR cleavage efficiencies range from 28–58%.

CONCLUSIONS

Electroporation is an efficient, fast and robust way to deliver many different types of macromolecules. Optimization of the pulse strength and selecting a universal electroporation solution can enable high efficiency electroporation in a broad range of cell types with minimal cytotoxicity post-electroporation.

FIGURE 6: Electroporation can effectively deliver siRNA and CRISPR RNP complexes. (A) siRNA and plasmid DNA were co-electroporated in the indicated cell lines with the Ingenio® electroporation solution in 0.2cm cuvettes using either the Gene Pulser Xcell™ Eukaryotic System (Bio-Rad). Plasmid encoding Firefly luciferase (10µg/mL) was co-electroporated with 250nM of either non-targeting control siRNA or GL3 siRNA into Jurkat E6-1, HeLa, or CHO-K1 cells. Twenty-four hours post electroporation, cells were harvested and assayed for luciferase activity. Data was normalized to the non-targeting control siRNA. (B) CRISPR ribonucleoprotein (RNP) complexes targeting WTAP, composed of 750nM Cas9 protein (NEB) and 1500nM pre-complexed two-part gRNA (IDT), was electroporated into K562 and Jurkat E6-1 cells using the Ingenio® Electroporation Solution (Mirus Bio) and a Gene Pulser Xcell™ Eukaryotic System (Bio-Rad). Exponential pulse conditions of 150V and 950µF for K562 or Jurkat E6-1 cells were applied to triplicate 0.2cm cuvettes, 100µL volume, 10 x 10^5 cells/mL +/- RNP complex. A T7E1 mismatch assay was used to measure cleavage efficiency at 48 hours post-transfection. Densitometry was used to quantitate the cleavage efficiency.

Easy optimization for high efficiency, low toxicity electroporation

INGENIO® EZPORATOR® ELECTROPORATION SYSTEM

- **Performance:** deliver any nucleic acid to hard-to-transfect stem and primary cells
- **Simplicity:** use a single, universal electroporation solution across all cell types
- **Flexibility:** easily optimize electroporation parameters for each cell type

The Ingenio® EZporator® Electroporation System was developed for efficiency, simplicity, and for use with the Ingenio® Electroporation Kits and Solution. Optimization is simple and protocols for various cell types are available. Every system comes with a complimentary Ingenio® Electroporation Kit (8 reactions, 0.4cm cuvettes) and an easy-to-use manual.

US ONLY

For more information, visit **vwr.com/mirusbio**

Description	Size	Cat. No.	Unit
Ingenio® EZporator® Electroporation System	—	**76304-532**	**Each**
Ingenio® Electroporaiton Soution and Kits			
Ingenio® Electroporation Solution	25 Reactions	**10766-836**	**Each**
Ingenio® Electroporation Solution	100 Reactions	**10766-848**	**Each**
Ingenio® Electroporation Kit with 0.2 cm Cuvettes	25 Reactions	**10766-838**	**Each**
Ingenio® Electroporation Kit with 0.2 cm Cuvettes	100 Reactions	**10766-850**	**Each**
Ingenio® Electroporation Kit with 0.4 cm Cuvettes	25 Reactions	**10766-840**	**Each**
Ingenio® Electroporation Kit with 0.4 cm Cuvettes	100 Reactions	**10766-990**	**Each**

At the time of publication, these products were not available in Canada. Please contact your VWR Life Science Specialist to learn about similar options available in your region.

PCR made easy and unstoppable

FAILSAFE™ PCR SYSTEMS FROM LUCIGEN

- Successful PCR, the first time and every time
- PCR amplification of difficult or high-GC templates
- Multiplex PCR compatible

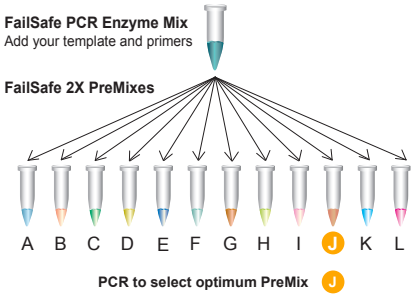
The FailSafe PCR System provides dependable, consistent high-fidelity PCR results for every DNA template, regardless of its source or sequence.

US ONLY

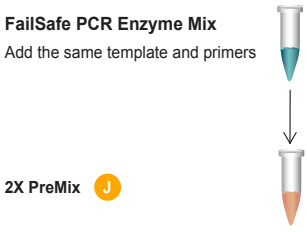
Description	Size	Cat. No.	Unit
FailSafe PCR PreMix Selection Kit (All 12 PreMixes)	60 Units	**76081-632**	**Each**
FailSafe Enzyme Mix Only	100 Units	**76081-640**	**Each**
FailSafe PCR 2X PreMix A	2.5 mL	**76085-636**	**Each**
FailSafe PCR 2X PreMix B	2.5 mL	**76085-638**	**Each**
FailSafe PCR 2X PreMix C	2.5 mL	**76085-640**	**Each**
FailSafe PCR 2X PreMix D	2.5 mL	**76085-642**	**Each**

At the time of publication, these products were not available in Canada. Please contact your VWR Life Science Specialist to learn about similar options available in your region.

1 Identify the optimal FailSafe 2X PreMix



2 Purchase and continue to use the optimal Failsafe 2X PreMix and PCR Enzyme Mix combination



HARDY DIAGNOSTICS
A Culture of Service™

There is always a better way

For more information, visit **vwr.com/hardy**

COMPACT DRY™ PLATE LINE

- Rigid transparent frame with removeable lid keeps your colonies intact when subculturing
- Self-diffusing dry media tranforms into gel with the 1mL sample so there's no need for an extra step
- Room temperature storage and stackable

The Compact Dry™ plate line presents a variety of organism-specific and total count tests, packaged up nicely in a sophisticated and sturdy cassette. This product line was designed for the end user with the idea of saving technicians time while performing high-throughput colony counts on food, beverage, cannabis, cosmetic, environmental, meat, raw material samples, and more.

Description	Application	Cat. No.	Unit
Compact Dry EC	E. coli and Coliforms	**10145-954**	**Pack of 100**
Compact Dry ETB	Enterobacteriaceae	**10753-986**	**Pack of 100**
Compact Dry LS	Listeria spp	**10789-408**	**Pack of 100**
Compact Dry SL	Salmonella	**10789-458**	**Pack of 100**
Compact Dry X-SA	Staphylococcus aureus	**10145-970**	**Pack of 100**
Compact Dry YM	Yeast and Mold	**10145-972**	**Pack of 100**
Compact Dry YMR	Yeast and Mold, Rapid	**76076-714**	**Pack of 100**
Compact Dry TC	Total Plate Count	**10145-968**	**Pack of 100**

Culturing hematopoietic cells serum-free

Lonza

Please visit **vwr.com** for additional sizes

TheraPEAK™ X-VIVO™ CELL CULTURE MEDIA FOR CELL AND GENE THERAPY

- Regulatory-friendly, serum-free cell culture medium
- High yield of viable cells within a short time
- Highly cited medium with research and clinical applications

The TheraPEAK™ X-VIVO™ medium is available in three different formulations. TheraPEAK™ X-VIVO™-10 is most suitable for culturing slow growing cells like stem cells. TheraPEAK™ X-VIVO™-15 is the most popular option with a formulation optimized for culturing immune cells like T cells and NK cells and TheraPEAK™ X-VIVO™-20 works best with quick grow growing cells e.g., PBL and TIL. The TheraPEAK™ X-VIVO™-15 and -10 are available with human and recombinant transferrin. All formulations listed below are 1 L.

Description	Formulation	Cat. No.	Unit
TheraPEAK™ X-VIVO™-10 Medium	with L-Glutamine, Gentamicin and Phenol Red	**12001-984**	**Each**
TheraPEAK™ X-VIVO™-10 Medium	with L-Glutamine, without Gentamicin and Phenol Red	**12002-004**	**Each**
TheraPEAK™ X-VIVO™-15 Medium	with L-Glutamine, Gentamicin and Phenol Red	**12001-988**	**Each**
TheraPEAK™X-VIVO™-15 Medium	with L-Glutamine, without Gentamicin and Phenol Red	**12002-006**	**Each**
TheraPEAK™X-VIVO™-20 Medium	with L-Glutamine, Gentamicin and Phenol Red	**12001-990**	**Each**

Break the 3D barrier

Get there fast with 3D cell culture

By Elizabeth Abraham, Sheila Carvalho, and Nicole Athanas

Corning can help you break through the barriers to creating more *in vivo*-like environments and predictive models — quickly and efficiently.

For more than 25 years, Corning has delivered innovations that have advanced the science of 3D cell culture. We pioneered the development of novel tools providing easier access to *in vivo*-like models, such as Corning Matrigel® matrix and Transwell® permeable supports. And we continue to support you with a diverse and evolving portfolio of innovative 3D cell culture products, solutions, protocols, and expertise. Corning is committed to working with you in critical areas like cancer biology, tissue engineering, and regenerative medicine — to help you bring safe, effective drugs and therapies to market in less time with greater certainty.

Whatever your application, we have the body of 3D cell culture knowledge and depth of resources to help you achieve your goals. It's no wonder so many scientists working in academic and biopharma labs look to Corning for solutions, guidance, and support when it's time to get started in 3D cell culture.

2D OR 3D? IT'S NO LONGER A QUESTION

Why have so many research scientists embraced 3D cell culture? Because cells grown in 3D more closely mimic *in vivo* behavior in tissues and organs than cells grown in a 2D culture model. 3D cell culture environments create more biologically relevant models for drug discovery which may lead to more predictive results, higher success rates for drug compound testing, a faster path to market, and reduced development costs.

Attribute	2D	3D
Growth Substrate	Rigid, inert	Mimics natural tissue environment
Cell Shape Growth	Loss of cell polarity and altered shape	Maintains *in vivo*-like morphology and polarity
Architecture	Not physiological, cells partially interact	Physiological, promotes close interaction between cells, ECMS, and growth factors
Growth Factor Diffusion	Rapid	Slow – biochemical gradients regulate cell-to-cell communication and signaling
Gene Expression	Different patterns of gene expression	Maintenance of *in vivo*-like expression patterns

CORNING 3D CELL CULTURE: DECADES OF EXPERIENCE WITH PROVEN RESULTS

Spheroid Microplates with ULA Surface

Corning spheroid microplates combine Corning's Ultra-Low Attachment (ULA) surface with an innovative well geometry to create an ideal tool for generating, culturing, and assaying 3D multicellular spheroids in the same microplate. The ULA coating attached to the interior surface of the Corning spheroid microplate well bottom enables highly reproducible growth of 3D cell spheroid cultures. 96-well and 384-well automation-friendly formats make it easier to generate spheroid models in a format suitable for high throughput screening (HTS) platforms.

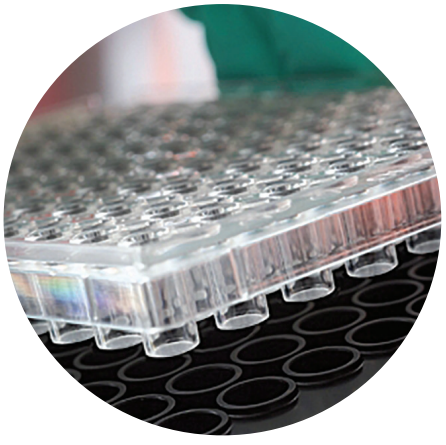
Matrigel® Matrix, ECMs, and Scaffolds

Corning® Matrigel matrix is an ECM-based hydrogel – proven and trusted since 1985 – that is suitable for a variety of cell types and functions. Matrigel matrix is a reconstituted basement membrane extract from Engelbreth-Holm-Swarm (EHS) mouse tumors and contains the prominent ECM molecules found in basement membrane. These components promote cellular functions that can support viability, proliferation, function, and development of many cell types, as well as subsequent cellular responses that are more physiologically relevant compared to cells grown in a 2D environment. Corning also offers other natural ECMs including collagen, laminin, and fibronectin.

Permeable Supports

Corning permeable supports are available in a variety of formats, pore sizes and membrane types. Transwell® and Falcon® permeable supports are widely used in complex cell culture models such as multi-layered tissues (skin, liver, kidney, human airway epithelia), migration/invasion assays, and co-culture applications. The unique design makes it possible to feed cells apically and basolaterally when growing cells to mimic the *in vivo* environment.

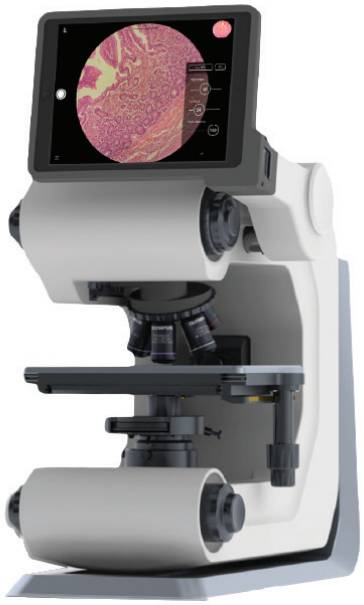
Description	Size	Cat. No.	Unit
Corning® Matrigel® Media			
Basement Membrane Matrix (GFR), Phenol Red-Free, LDEV-Free	10 mL	**47743-722**	**Each**
Basement Membrane Matrix (GFR), LDEV-Free	10 mL	**47743-720**	**Each**
Basement Membrane Matrix, LDEV-Free	10 mL	**47743-715**	**Each**
Corning® Ultra-Low Attachment Spheroid Microplates			
Black with Clear Round Bottom Microplates, Individually Packed, with Lid, Sterile	96-Well	**10037-558**	**Cs. 50**
Black Clear Round Bottom Microplates, Bulk Packed 10 per Bag, with Lid, Sterile	384-Well	**10037-556**	**Cs. 50**
Costar® Transwell® Permeable Supports			
Permeable Support PC Membrane, Sterile	6.5 mm Insert/8.0 µm Pore Size	**29442-120**	**Cs. 48**
Permeable Support with PET Membrane, Sterile	6.5 mm Insert/0.4 µm Pore Size	**29442-082**	**Cs. 48**
Permeable Support with PET Membrane, Sterile	12 mm Insert/0.4 µm Pore Size	**29442-078**	**Cs. 48**



Understanding microscopy basics

Configurations for core applications

By Eugene Cho



Understanding the fundamentals of various microscope configurations can help ensure that the appropriate tools are being used to address the variety of research needs within a lab.

UNDERSTANDING MICROSCOPY BASICS: CONFIGURATIONS FOR CORE APPLICATIONS

Microscopes have become indispensable tools essential for research, discovery, and education. While these instruments can accommodate highly specialized applications, there are four basic configurations that are critical to the optimum performance of almost every microscope.

OPTICAL CONFIGURATIONS: UPRIGHT VS INVERTED

1) The Upright Microscope

The Upright microscope is the oldest and most widely used configuration where the objective lenses are located above the sample. The configuration of the objective lens above the sample is ideal to view glass slides with coverslip mounts (Figure 1).

To illuminate the sample, a high N.A. (Numerical Aperture) or high-resolution condenser is placed just below the slide (Figure 2). The close proximity of this high N.A. condenser provides ideal illumination conditions that maximize resolution and reduce optical aberrations.

When using high resolution oil objectives on an Upright, a larger area can be covered with immersion oil. With the oil sitting

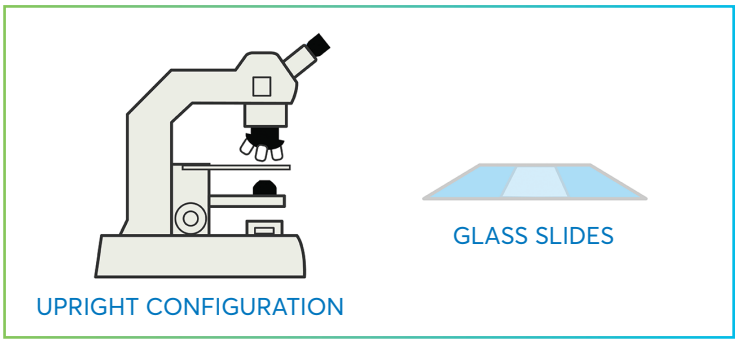


FIGURE 1: Upright microscope

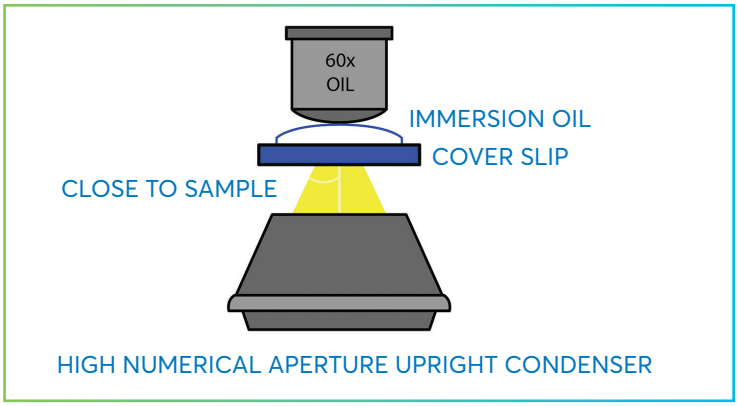


FIGURE 2: Condenser upright

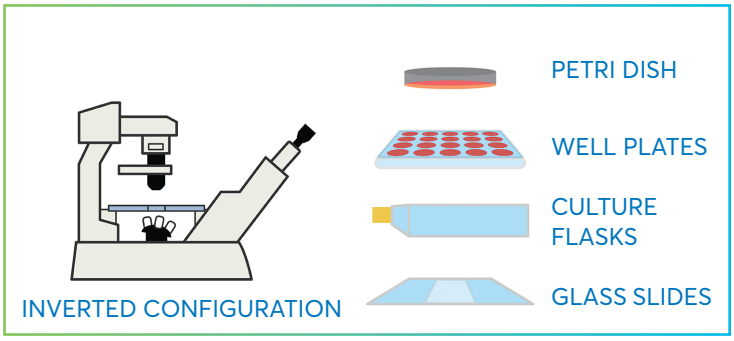


FIGURE 3: Inverted microscope

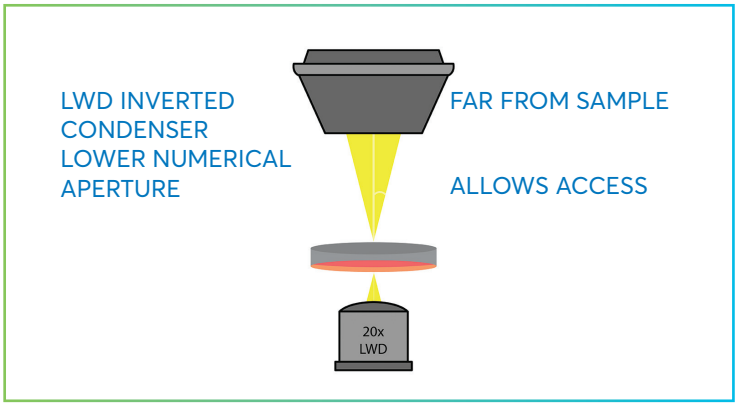


FIGURE 4: Condenser inverted

on the upward facing coverslip, it is easier to apply the oil, and there is less risk for contamination of other objectives or critical components.

As this configuration was optimized to view glass slides, upright microscopes are not well suited for viewing well plates, culture dishes and flasks.

2) The Inverted Microscope

The Inverted microscope places the objectives below the sample (Figure 3). They are frequently used to view live samples in plastic chambers such as well plates, culture dishes and flasks. Because these samples require aqueous media, they are almost always imaged from the bottom.

Long Working Distance (LWD) Objectives and Condensers are required to work with these thicker plastic chambers (Figure 4) The Inverted also allows users to view and access their samples for splitting cells, picking colonies, or changing media. However, these longer working distances optics compromise resolution

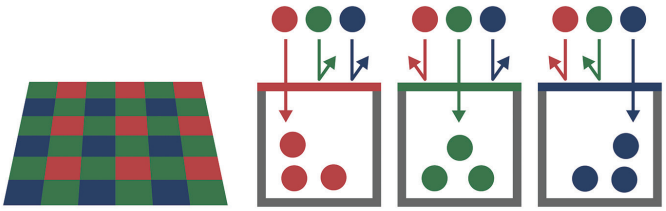


FIGURE 5: Color CMOS sensors work by using a Bayer Mask.

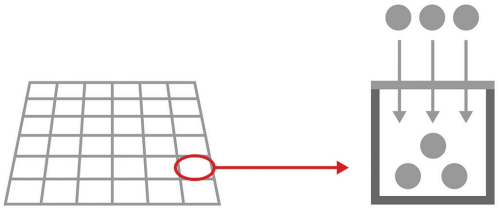


FIGURE 6: Monochrome CMOS sensor is much better for fluorescence microscopy.

as N.A. (Numerical Aperture) must be sacrificed for increased working distance.

While inverted microscopes offer greater flexibility with various sample chambers, they can encounter difficulties when working with glass slides. With the slide facing down and the coverslip facing towards the objective, protruding labels or excessive mounting media can interfere with the slide lying flat and create a "rolling focus". Also, immersion oil can easily contaminate other objectives and critical optical components as the oil will fight gravity to stay in place.

CAMERA CONFIGURATIONS

Digital cameras are widely used in microscopy today. Biological samples can present both beautiful images and quantitative data, and there are multiple imaging approaches that leverage various sensors on the market today. The requirements of Brightfield imaging are significantly different than those of fluorescence. Using the right camera for the correct application is critical for both image quality and data integrity.

3) COLOR CMOS Camera: Brightfield

All CMOS sensors are inherently monochromatic (black and white). One method of adding color capability is to apply alternating red, green, and blue color filters directly over each pixel – also known as the Bayer Mask (Figure 5). As a single shot is captured, the red, green, and blue pixel values are averaged and interpolated to create a color image. A known tradeoff is that the effective resolution of the camera sensor is reduced because it's pixels are divided into red, green, and blue components.

Consumer cameras today mitigate these effects by increasing the number of pixels in the sensor. In microscopy, these color CMOS cameras are widely accepted and commonly used to capture Brightfield images.

4) Monochrome CMOS Camera: Fluorescence

Fluorescence microscopy requires a light sensitive camera to perform under low light conditions. The Bayer Mask interferes

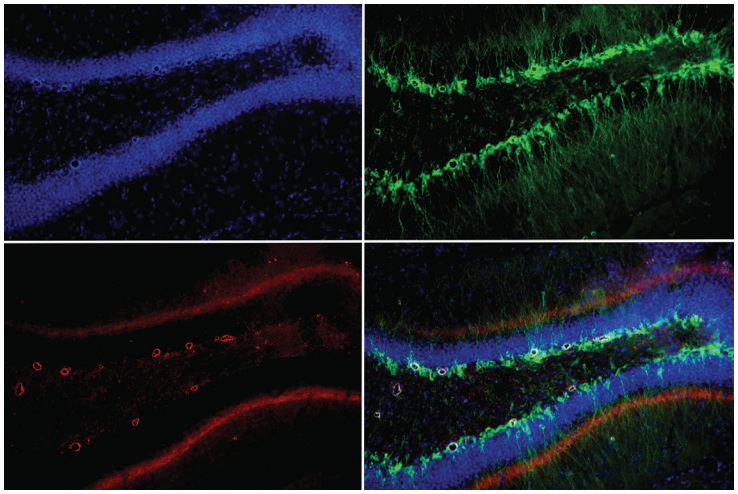


FIGURE 7: Fluorescence overlay captured with monochrome CMOS camera.Agilent Technologies — building upon Stratagene's legacy of excellence — provides a wide range of high-fidelity, specialty, and routine molecular biology tools to meet all of your research needs.

and significantly reduces the transmission of light photons to the camera sensor by 60-80%. A monochrome sensor, without the Bayer Mask, is much more efficient at capturing light. This higher quantum efficiency (QE%) is paramount for capturing high quality images in fluorescence microscopy.

Additionally, a Bayer Mask sensor should not be used for fluorescence imaging because it averages and interpolates pixels to form a color image. This image processing results in data that is altered and compromised. Therefore, quantitative data for intensity analysis can only be obtained with a monochrome camera (Figure 6). Fluorescence images can still be viewed in color by adding "pseudo-color" in software for each channel (Figure 7).

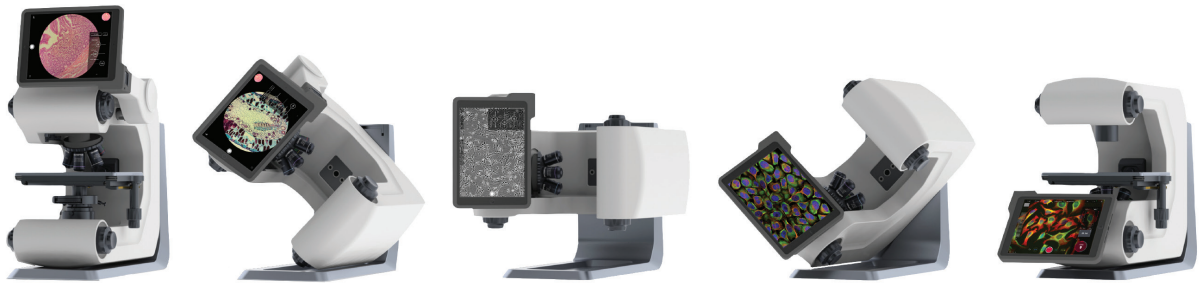
UNDERSTANDING THE BASICS

Microscopes can be considerable investments that are capable of producing high quality images, addressing a wide array of samples. Understanding the fundamentals of these configurations can help to ensure that the appropriate tools are being used to address the variety of research needs within a lab.

The world's first and only hybrid microscope

ECHO REVOLVE MICROSCOPE

- Four modes include Upright + Inverted + Brightfield + Fluorescence
- Ultra high-resolution 13" Retina Touch Display driven by intuitive software
- Two instruments for the price of one, saving funds and benchspace
- Features a familiar app interface with responsive touch control



Description	Cat. No.	Unit
REVOLVE4 Upright, Inverted, Brightfield, Fluorescent Microscope	76299-574	Each

Agilent Technologies

Agilent Technologies genomics portfolio is now available at VWR!

Visit **vwr.com/agilent** to learn more

- **sgRNA and CRISPR pooled libraries:** custom and catalog options available
- **Complete qPCR and PCR solutions:** instruments (AriaMx for qPCR and SureCycler for standard PCR) and best in class reagents available in component or mastermix formats
- **Mutagenesis and Cloning kits:** industry leading QuickChange kits and innovative competent cell strains
- **Multiple Affinity Removal System (MARS):** immunodepletion columns for proteomics and protein discovery

Endothelial cell culture

By Christian Gojak, PromoCell

The *in vitro* culture of primary human endothelial cells is a widely established model system used to investigate several cardiovascular diseases and cancer research applications. Our comprehensive endothelial cell culture portfolio consists of 12 different types of large vessel and microvascular human endothelial cells with optimized growth media for each cell type.

TISSUE SOURCES AND DONOR INFORMATION

PromoCell large vessel endothelial cells are available from the umbilical vein and artery, the aorta, the coronary artery, the pulmonary artery, and the saphenous vein. Our microvascular endothelial cells are available from dermal, lung, cardiac and uterine tissues. All our cell types are sourced from healthy, consented donors. Specific donor information like age, sex and tissue location is available for each produced lot. Endothelial cells from diseased donors (e.g., diabetes, myopathies) are available upon request.

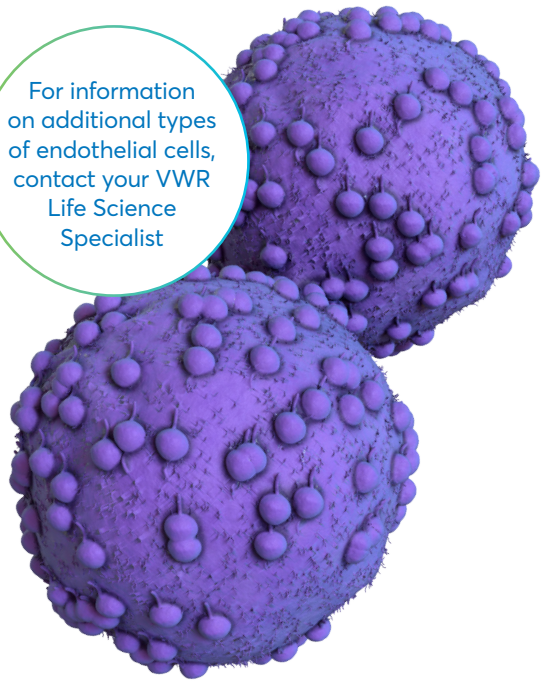
QUALITY CONTROL

Each produced cell lot is tested for cell morphology, adherence rate, cell viability, flow cytometric analyses for cell-type specific markers, e.g., von Willebrand Factor (vWF) and CD31, as well as Dil-Ac-LDL uptake. Growth performance is tested through multiple passages up to 15 population doublings (PD) under culture conditions without antibiotics and antimycotics. In addition, all cells have been tested for the absence of HIV-1, HIV-2, HBV, HCV, HTLV-1, HTLV-2 and microbial contaminants (fungi, bacteria, and mycoplasma).

CELL CULTURE MEDIA – OPTIMIZED FOR EACH CELL TYPE

Just like the cellular microenvironment is different for large and microvascular vessels, such are their media requirements. At PromoCell we have developed optimized growth media for both large vessel and microvascular endothelial cell types. In addition, we offer media formulations with and without Endothelial Cell Growth Supplement (ECGS), a bovine hypothalamic extract traditionally used for cultivating endothelial cells without added VEGF. Generally, VEGF leads to higher endothelial cell proliferation in culture. But, because of its multiple effects on the cell metabolism, it may also interfere with certain experimental setups.

For information on additional types of endothelial cells, contact your VWR Life Science Specialist



Description	Cat. No.	Unit
Human Primary Endothelial Cells — 500,000 Cryopreserved Cells		
Human aortic endothelial cells (HAoEC)	**10171-970**	**Each**
Human umbilical vein endothelial cells (HUVEC), pooled donors	**10171-910**	**Each**
Human umbilical vein endothelial cells (HUVEC), single donor	**10171-906**	**Each**
Human coronary artery endothelial cells (HCAEC)	**10171-940**	**Each**
Endothelial Cell Growth Media, 500 mL		
Endothelial Cell Growth Medium Kit	**10172-288**	**Each**
Endothelial Cell Growth Medium MV Kit	**10172-292**	**Each**
Endothelial Cell Growth Medium MV 2 Kit	**10175-256**	**Each**
Endothelial Cell Basal Medium, Phenol Red-Free	**10175-266**	**Each**

Monitoring tumor progression using the iBox® Scientia™ Imaging System

By Tony Sanchez, MD, Life Scientist, UVP, LLC, Upland, CA

Molecular imaging offers benefits over *ex vivo*, cross-sectional techniques like immunohistochemistry or H&E staining due to the non-destructive nature of fluorescence imaging

By preserving the native environment of the target, repeated experimentation of a particular animal or tissue sample can be conducted. With the appropriate reporter, such as green fluorescent protein (GFP) or red fluorescent protein (RFP), molecular imaging can localize a biological phenomenon to a particular location and track that process over time. Thus, by targeting a gene with a genetic reporter such as fluorescent proteins or downstream expression with a fluorophore conjugate, *in vivo* fluorescence imaging can yield anatomical, functional and molecular data.

Molecular imaging has many applications that span multiple therapeutic areas. One application with great utility in cancer biology is the tracking of tumor lesions implanted within mice. Monitoring of tumor growth over time is a useful tool for observing the efficacy of chemotherapeutic agents in either shrinking or stabilizing tumor lesions. In a recent experiment, mice were implanted with a cancer cell line expressing fluorescent proteins and imaged on a weekly basis. Through the use of the iBox Scientia Imaging System (UVP, LLC), tumor lesions can be visually tracked and quantified with a high quantum efficiency camera, powerful light source, quality lenses and interchangeable filter sets to image fluorescence across a wide spectral range from 450 nm to near infrared (NIR being 700nm and greater).

MATERIALS AND METHODS

Four adult nude female mice (AntiCancer, Inc., San Diego, CA) were injected with $3x10^6$ human colon HCT-116 dual-color cancer cells directly into the peritoneal cavity. Once the implanted tumor lesions reached 1mm in diameter, all mice were monitored and GFP/RFP fluorescence images were captured weekly for a total of six weeks to monitor the growth of the implanted cancer cells.

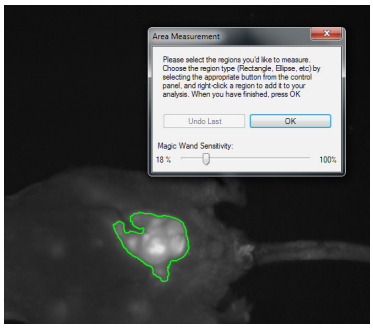


FIGURE 1: Snapshot of area density measurement of tumor mass. The highlighted area is a luminescent lesion composed of GFP and RFP-expressing cells roughly 1cm in diameter. VisionWorksLS software automatically selects for the brightest region and, with calibration, can yield quantitative information regarding light density.

Two color channels were selected to image the growth of tumors within each mouse. All samples were excited using the 150W halogen BioLite™ MultiSpectral Light Source (UVP, LLC) and color-specific excitation filters. A blue excitation filter with a peak wavelength of 475nm and a bandpass of 40nm (475/40) was used to excite GFP. A green excitation filter (525/45) was used to excite RFP. To select for green fluorescence, an emission filter with a peak wavelength of 535nm and a bandpass of 44nm (535/44) was used. The red channel employed the 605/50 filter. Images were captured using the BioChemi 500, a 4.2 MP cooled monochrome CCD camera (UVP, LLC).

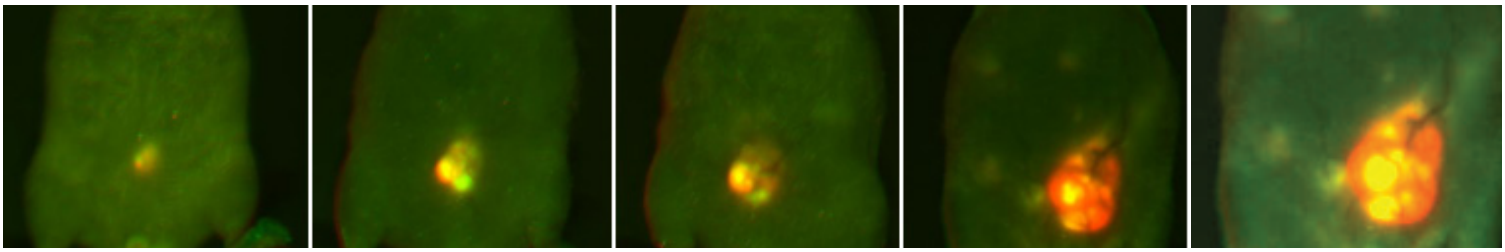


FIGURE 2: **Highlighting the progressive increasing luminosity of an implanted lesion in the peritoneum.** Each image was captured using both GFP and RFP filters and then multiplexed. The intense fluorescent signal shown in the implanted tumor is significantly brighter than the surrounding tissue. Note the formation of metastatic lesions beginning to develop distal to the site of implantation and becoming visible within the abdomen in the final slide.

Images captured using the BioChemi 500 camera were acquired and multiplexed using VisionWorks®LS software (UVP, LLC). Background fluorescence was removed using histogram adjustment and monochrome images were pseudocolored according to the emission specifications (RFP and GFP). Images were multiplexed in order to overlay multiple images. Area density measurements were acquired using multiplexed monochrome images. Finally, tumor size was determined by taking the square of the pixel density for each lesion at a given time interval (Figure 1).

RESULTS

Figure 2 shows the progression of one tumor lesion over the course of several weeks. In the last image, metastatic lesions are observed emerging within the peritoneum (smaller areas of fluorescence).

Pooled data for tumor measurements can be seen in Figure 3. Tracking the lesions over time reveals a relatively linear increase in tumor burden with an average maximum area density of 7.4 X 10^3 pixels squared.

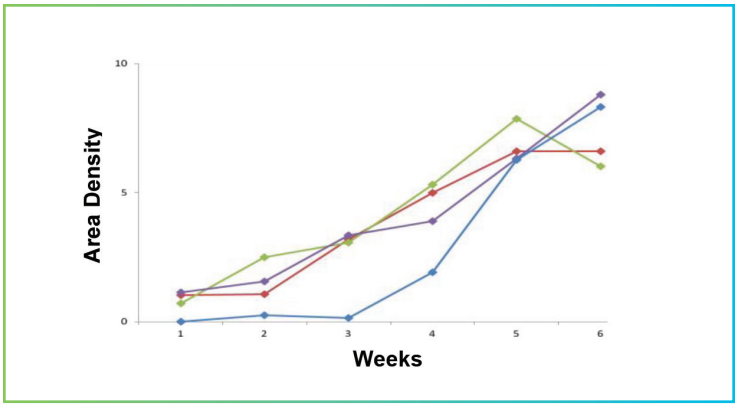


FIGURE 3: **Graphical representation of area density of tumor lesions over time of the implanted mouse population.** All four mice show increase in tumor burden over time (weeks along the x-axis) as measured by light density (pixels squared X 10^3 along the y-axis). Each point represents a single lesion measurement or pooled area density of multiple lesions.

CONCLUSION

In this experiment, implanted colon cancer cells, HCT-116, were tracked over time within the abdominal cavity of mice. The growth of tumors showed a steady increase in area density within all four mice, reaching a peak at six weeks. This steady increase in growth was measured using the area density function of VisionWorksLS software, and correlates strongly with volume (2003).

The ability to non-invasively monitor functional or molecular changes offers advantages over immunohistochemical and other cross-sectional techniques due to the non-destructive nature of *in vivo* imaging. A disease process can be tracked by simply transfecting a cell or organism with a fluorescent protein or tagging a downstream molecule with a fluorophore conjugate and monitoring the changes in real time.

The iBox Scientia is developed to image fluorescence-labeled cells by maximizing signal-to-noise through the use of a high resolution cooled CCD camera, highly sensitive optics, a directed illumination source and a large selection of excitation and emission filters. Given the breadth of fluorescent proteins and fluorophores available to researchers, applications using the iBox in pre-clinical studies include, but are not limited to, cancer biology, cardiovascular disease and immunology.

REFERENCES

1. Katz MH, Takimoto S, Spivak D, Moosa AR, Bouvet M. 2003. J Surg Res. 151-160.

US ONLY

Description	Cat. No.	Unit
UVP iBox Scientia 615, 50 mm	**89237-432**	**Each**
UVP iBox Scientia 900, 25 mm	**76288-262**	**Each**
UVP iBox Scientia 900, 50 mm	**76288-264**	**Each**
UVP iBox Explorer2 Imaging Microscope	**89237-430**	**Each**
UVP iBox Studio touch, NIR, 615	**76299-404**	**Each**
UVP iBox Studio touch, NIR, 815	**76299-396**	**Each**

At the time of publication, these products were not available in Canada. Please contact your VWR Life Science Specialist to learn about similar options available in your region.

Innovative power supplies for nucleic acid and protein electrophoresis applications

VWR® 300V AND 500V POWER SUPPLY

- Color touch screen control panel
- Compact footprint, takes up minimal lab space
- Four sets of output terminals compatiable with all popular gel tanks
- Ideal for horizontal DNA gels, vertical protein gels

Specifications

Voltage Output	300V (1 volt increments)	500V (1 volt increments)
Current Output	2000mA (5 mA increments)	400mA (1 mA increments)
Output Control	Constant voltage or constant current	Constant voltage or constant current
Maximum Power	300W	120W
Output Terminals	4 pairs	4 pairs
Electrical Input	115V / 60Hz or 230V / 50Hz	115V / 60Hz or 230V / 50Hz
Weight/Dimensions	1.2 kg (2.7 lbs)/ 8.9 x 12.7 x 21.6 cm (3½ x 5 x 8½")	1.14 kg (2.5 lbs) / 8.9 x 12.7 x 21.6 cm (3½ x 5 x 8½")

Description	Cat. No.	Unit
300V, 115 VAC/60Hz Input	**76196-458**	**Each**
300V, 230 VAC/50Hz Input	**76196-460**	**Each**
500V, 115 VAC/60Hz Input	**76196-454**	**Each**
500V, 230 VAC/50Hz Input	**76196-456**	**Each**

VWR® MINI POWER SUPPLY

- Compact design, fits almost anywhere
- Simple operation — connect gel box and select voltage to start
- Selectable, 100V or 200V output
- Ideal for basic DNA separations in agarose gels
- Compatible with standard horizontal gel boxes
- Two pairs of output jacks for running two gels at one time

Specifications

Voltage Output	100V or 200V, constant
Current Output	Maximum 200mA
Maximum Power	40 Watts
Output Terminals	2 pairs in parallel
Electrical Input	115V / 60 Hz or 230V / 50 Hz
Weight/Dimensions	0.4 kg (0.9 lbs.)/ 16 x 11.5 x 5.7 cm (5¼ x 4½ x 2¼")
Warranty	2 years

Description	Cat. No.	Unit
115 VAC/60 Hz Input	**76196-450**	**Each**
230 VAC/50 Hz Input	**76196-452**	**Each**

MOLECULAR DEVICES

Affordable, automated cellular imaging

NEW

Visit **vwr.com/molecular-devices** to learn more

ImageXpress™ PICO AUTOMATED CELL IMAGING SYSTEM

Compact, simple, and affordable – the ImageXpress™ Pico system shortens the learning curve, so you can start running experiments quickly.

- Go from samples to results in minutes
- 20+ preconfigured protocols for cell counting, neurite tracing, fluorescence imaging, and more
- Integrated data visualization tools

Description	Filter Type	Includes	Cat. No.	Unit
ImageXpress Pico Basic Bundle	FITC (Filter Cubes)	FLUOTAR 4x/NA 0.13 and 10x/NA 0.32 Objectives	**76230-060**	**Each**
ImageXpress Pico Advanced Bundle	FITC DAPI TRITC Cy5 (Filter Cubes)	FLUOTAR 4x/NA 0.13, FLUOTAR 10x/NA 0.32, FLUOTAR 20x/NA 0.40 and FLUOTAR 40x/NA 0.60 Objectives	**76230-062**	**Each**

For Research Use Only. Not for use in diagnostic procedures.

Fast, reliable DNA purification for NGS workflows

Quantabio

For more information, visit **vwr.com/quantabio**

sparQ PureMag BEADS

- High recovery of DNA fragments greater than 100 bp
- Efficient removal of unwanted components from adapter ligation and PCR reactions
- Consistent single or double-sided size selection
- Seamless integration into existing NGS workflows with little or no protocol change
- Easy-to-use and compatible with manual processing or automated liquid handling robots

sparQ PureMag Beads is a fast and reliable nucleic acid purification system for reaction cleanup and size selection in Next Generation Sequencing (NGS) workflows. Based on the reversible nucleic acid-binding properties of magnetic beads, this product can be used to quickly remove primers, primer-dimers, unincorporated nucleotides, salts, adapters, and adapter-dimers from NGS library prep reactions to improve downstream sequencing performance.

Description	Size	Cat. No.	Unit
sparQ PureMag Beads	5 mL	**76302-834**	**Each**
sparQ PureMag Beads	60 mL	**76302-832**	**Each**
sparQ PureMag Beads	450 mL	**76302-830**	**Each**

Optimized reagents for robust T7-endonuclease-based detection of genome editing events

Visit **vwr.com/neb** for more information

EVALUATE TARGETING EFFICIENCY WITH THE ENGEN® MUTATION DETECTION KIT AND T7 ENDONUCLEASE I

- Includes control template and primer mix for amplification and digestion reactions
- No purification prior to digestion required
- Digestion reaction complete in 15 minutes

The T7 Endonuclease I mutation detection assay is widely used for identification of mutations. This assay detects heteroduplex DNA that results from the annealing of a DNA strand, including desired mutations, with a wild-type DNA strand. The EnGen Mutation Detection Kit provides optimized reagents for performing robust T7 Endonuclease-based detection of genome editing events.

Description	Size	Cat. No.	Unit
EnGen Mutation Detection Kit	25 Reactions	**103049-174**	**Each**
T7 Endonuclease I	250 Units	**101228-382**	**Each**
T7 Endonuclease I	1,250 Units	**101228-380**	**Each**

Rapid, simple synthesis of sgRNA for CRISPR experiments

GENERATE MICROGRAM QUANTITIES OF sgRNA IN LESS THAN ONE HOUR WITH THE ENGEN® sgRNA SYNTHESIS KIT, *S. pyogenes*

- Eliminates separate template preparation and IVT steps
- Template synthesis and transcription combined in a single-tube reaction
- Synthesis complete in an hour or less
- High-quality sgRNAs can be used directly in downstream applications

The EnGen sgRNA Synthesis Kit simplifies the generation of microgram quantities of custom sgRNAs in an hour or less by combining template synthesis and transcription. sgRNAs are suitable for downstream applications, including CRISPR/Cas-9-based genome editing and *in vitro* DNA cleavage.

Description	Size	Cat. No.	Unit
EnGen sgRNA Synthesis Kit, S. pyogenes	20 Reactions	**103218-922**	**Each**

Immune checkpoints and co-stimulatory reagents

Facilitate your regulation study

By Raphael Levi, PhD

The recognition of the antigenic peptide-MHC I/II complex on antigen presenting cells (APCs) through the T cell receptor (TCR) initiates T cell-mediated immunity. However, the interaction of antigen-MHC complexes with the TCR is not sufficient for activation of naïve T cells. Additional costimulatory signals are required for T cells to become fully activated.

Once activated, T cells proliferate and migrate to sites of inflammation where they attack cells expressing relevant antigens, and destroy them either directly or indirectly through other effector cells or factors (e.g., cytokines). Effective immune response is imperative to defend against invading pathogens, as well as against malignant neoplasms; however, dysregulated immune response can result in chronic inflammation, which leads to tissue damage and autoimmunity.

Immune checkpoints are inhibitory regulators of the immune system that are crucial to maintaining self-tolerance, preventing autoimmunity, and controlling the duration and extent of immune responses in order to minimize collateral tissue damage. These immune checkpoints are often overexpressed on tumor cells or on non-transformed cells within the tumor microenvironment, and compromise the ability of the immune system to mount an effective anti-tumor response.

Immune responses are regulated by a balance between co-stimulatory and inhibitory signaling pathways, which are stimulated by an assortment of receptors and their respective ligands (Figure 1). The ability to shift the balance towards a desired response can offer a way to ameliorate a variety of diseases.

The blockade of immune checkpoints is among the current most promising approaches for activating therapeutic anti-tumor immunity and circumventing the immune resistance exhibited by many tumors. The first immune checkpoint therapy approved by the FDA targeted the Cytotoxic T Lymphocyte Associated antigen 4 (CTLA4) pathway (Ipilimumab, trade name Yervoy, Bristol-Myers Squibb). Subsequently, the FDA approved two treatments targeting the programmed cell death protein 1 (PD-1) pathway (Pembrolizumab, trade name Keytruda, Merck; and Nivolumab, trade name Opdivo, Bristol-Myers Squibb)(Mahoney 2015).

Improved understanding of immunomodulatory pathways spurred the study of numerous immune targets as potential anti-cancer therapies, including studies that investigate the synergistic effect of combining such therapies.

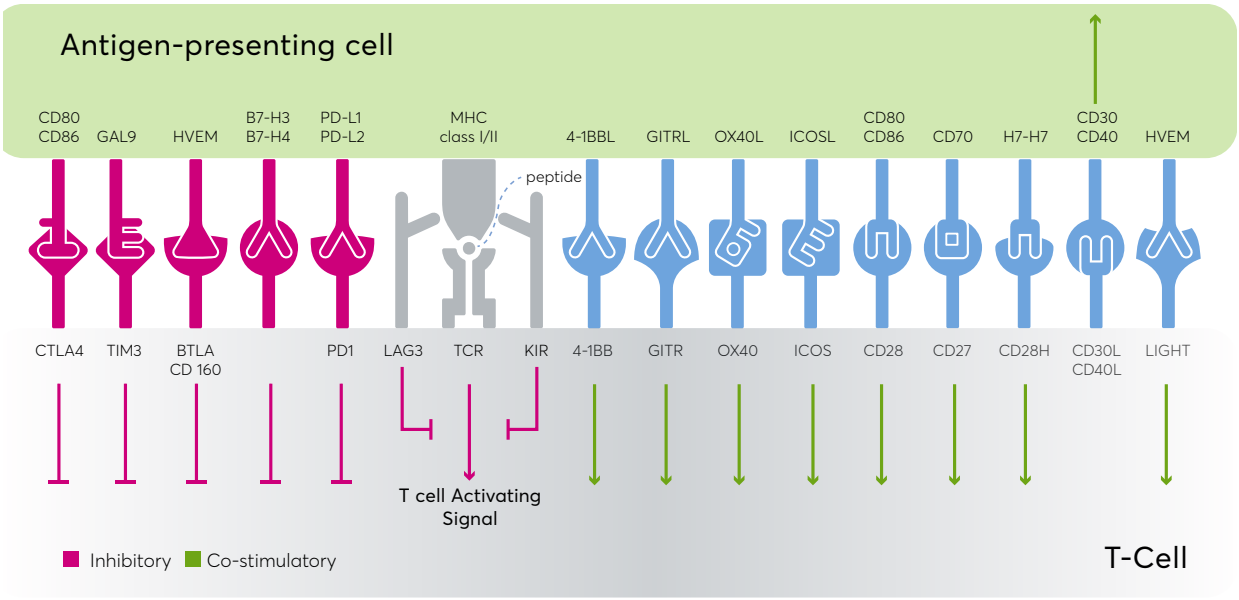


FIGURE 1: Co-signaling interactions in T cells. Co-stimulatory molecules deliver positive signals to T cells following their binding to ligands and receptors on APCs. Inhibitory (Checkpoint) molecules deliver negative signals to T cells upon interaction with their counterparts on the APCs.

The robust anti-tumor responses and tumor regression that are achieved by targeting immune checkpoint pathways suggests that the opposite strategy, of engaging these molecules, may alleviate inflammation in autoimmune diseases. This approach is supported by the FDA approval of the humanized fusion protein CD152-IgG1 (Abatacept, trade name Orencia, Bristol-Myers Squibb). Abatacept inhibits T cell activation by selectively blocking the co-stimulatory signal induced by interaction of CD80/CD86 ligands with CD28, which in turn inhibits T cell proliferation and B cell immunological response.

In the future, the examination of immune infiltration and expression patterns of immunoinhibitory markers will be used to optimize treatment to combine both checkpoint blockers and immune agonists.

THE B7-CD28 SUPERFAMILY

The B7 family of ligands and the CD28 family of receptors belong to the Immunoglobulin SuperFamily (IgSF). Interactions between B7 ligands and CD28 receptors play an essential role in regulating T cell response by eliciting both positive co-stimulatory and negative inhibitory signals. There are currently ten known members of the B7 family: B7-1 (CD80), B7-2 (CD86), B7-H2 (ICOSL), programmed death ligand 1 (PD-L1), programmed death ligand 2 (PD-L2), B7-H3, B7-H4, B7-H5, B7-H6, and B7-H7; and five known members of the CD28 family: CD28, CTLA-4, ICOS, PD-1, and B- and T-Lymphocyte Attenuator (BTLA).

CD28 and B7-1/B7-2

CD28 is the only B7 receptor that is constitutively expressed on naïve T cells and provides co-stimulatory signals, which are required for T cell activation and survival. Interaction of CD28 and its ligands, B7-1 (CD80) and B7-2 (CD86), is involved in T cell activation, stimulation of cell proliferation and cytokine production, and promotion of T-cell survival. B7-1 expression is upregulated in APCs when activated by Toll-like receptor ligands, while B7-2 is constitutively expressed on APCs.

ICOS and ICOSL

ICOS is expressed on activated T cells, while its ligand, ICOSL, is expressed on APCs such as B cells, macrophages, monocytes, and dendritic cells (DCs). The interaction of ICOS with ICOSL mediates T cell activation and expansion, is involved in T cell dependent B cell activation, and Th cell differentiation.

CTLA-4 and B7-1/B7-2

CTLA-4 expression is up¬regulated rapidly following T cell activation and CD28 ligation on the surface of T cells. It is also expressed on regulatory T cells (Tregs) as well as on some types of non-T cells, both normal and malignant. CTLA-4 and CD28 share the same B7 family ligands, B7-1 and B7-2, which are both expressed on APCs; however, CTLA-4 has a 20–100-fold higher affinity than CD28. Ligation inhibits cell proliferation, cytokine production and cell cycle progression. CTLA-4 competes with CD28 for B7-1/B7-2 ligands, thereby blocking the CD28 co-stimulatory signal that is necessary for robust T cell activation and effector function. An

immunotherapy treatment based on blocking the inhibitory effect of CTLA-4 has been approved by the FDA.

PD-1 and PD-L1/PD-L2
PD-1 is primarily expressed on activated T cells, natural killer (NK) cells, B cells and certain myeloid cells. When activated, PD-1 signals to negatively regulate the immune response. There are two known ligands of PD-1: PD-L1 and PD-L2. PD-L1 is expressed on numerous immune cell types, including T cells, B cells, DCs, and on many tumor cells; while PD-L2 expression is induced on DCs and macrophages by IL-4, LPS and INF-γ (Nguyen 2015). PD-1 plays an important role in reducing autoimmunity and promoting self-tolerance by preventing the activation of T-cells and down regulating immune responses. PD-1 induced inhibition is achieved through promoting apoptosis in antigen specific T cells in lymph nodes, while simultaneously reducing apoptosis in Tregs. Research has shown that tumors exploit PD-1 signaling to evade immune detection, the importance of which is highlighted by the FDA's approval of immunotherapy treatments using PD-1 signaling blockers to induce antitumor response.

CD28H and B7-H7
CD28H (CD28 homolog) is widely expressed, mainly by epithelial and endothelial cells. It is also expressed in lymphoid organs and in peripheral blood mononuclear cells, such as NK cells and CD3+ T cells. Its ligand, B7-H7, is highly expressed in B-cells, dendritic cells, monocytes and macrophages, but not in T cells. The interaction of CD28H with B7-H7 selectively co-stimulates human T cell growth and cytokine production in the context of TCR-mediated activation.

TIM-3 and GAL9
TIM-3 (T cell Immunoglobulin Domain and Mucin Domain 3), which belongs to the IgSF, is highly expressed on Th1 lymphocytes and CD11b+ macrophages and is upregulated on activated T and myeloid cells. TIM-3 regulates macrophage, activation and inhibits Th1 mediated immune responses to promote immunological tolerance. One of its ligands is GAL9 (Galectin-9), which belongs to the galectin family of lectins. The binding of GAL9 to TIM-3 can negatively regulate Th1 immune response, enhance immune tolerance and inhibit anti¬tumor immunity. Dysregulation of the TIM-3/GAL9 pathway is implicated in many chronic autoimmune diseases, such as multiple sclerosis and systemic lupus erythematosus.

KIR and MHC-I
KIRs (Killer cell immunoglobulin-like receptors), a family of receptors belonging to the IgSF, are expressed on NKs and subset of T cells. KIRs regulate the killing function of these cells by interacting with MHC-I molecules, which are expressed on all cells types. Most KIRs inhibit the cytotoxic activity of NKs upon interaction.

B7-H3
B7-H3 (B7 homolog 3) from the B7 family of the IgSF, is predominantly expressed on professional APCs, including B cells, macrophages, DCs and a wide variety of human cancer cells. It is also expressed on a broad variety of non-immune cells, suggesting additional non-immunological functions. Both stimulatory and inhibitory properties have been identified but the ligand of B7-H3 has yet to be identified.

B7-H4
B7-H4 (B7 homolog 4), from the B7 family of the IgSF, is expressed on the surface of activated lymphocytes, macrophages, monocytes, DCs, epithelial cells, and bone marrow-derived mesenchymal stem cells, as well as numerous tumor cells. It is a negative regulator of T cell immunity that inhibits T cell proliferation, cytokine production, and cell cycle progression. Its ligand is still unknown.

LAG-3 and MHC-II
LAG-3 (Lymphocyte-activation gene 3) is expressed on activated T cells, NKs, B cells, and plasmacytoid DCs. Its main ligands are MHC-II molecules, which are constitutively expressed on professional immune APCs. LAG-3 binds MHC-II in a similar manner to CD4, but with higher affinity. It negatively regulates proliferation, activation, and homeostasis of T cells in a similar fashion to CTLA-4 and PD-1.

TUMOR NECROSIS FACTOR

Receptor Superfamily (TNFRSF)
The TNFRSF is comprised of the homologs, and currently includes 29 receptors, which bind ligands via an extracellular cysteine-rich domain, and 19 ligands. Most TNF receptors form trimeric complexes in the plasma membrane upon activation and require an adaptor protein for downstream signaling. TNF receptors are mainly involved in apoptosis and inflammation, but can also participate in other signaling pathways, such as proliferation, survival and differentiation. Several of the members of this family contain cytoplasmic 'death domains' that are crucial for the initiation of an apoptotic response.

HVEM and LIGHT/BTLA /CD160
HVEM (TNFRSF14, Herpes Virus Entry Mediator) is a member of the TNF receptor superfamily that is widely expressed on APCs, endothelium and lymphocytes, especially on naïve T cells. It serves as a receptor to several ligands, including LIGHT, TNF-, BTLA (B and T lymphocyte attenuator), and CD160. Expressed by activated T cells, NK cells, monocytes, granulocytes and immature dendritic cells, LIGHT functions as a costimulatory factor for the activation of lymphoid cells when binding to

HVEM. In contrast to LIGHT, BTLA and CD160 inhibit of T cell activation when bound to HVEM. BTLA is expressed by splenic B and T cells, macrophages, NK cells and dendritic cells; whereas, CD160 is expressed by NK cells, NKT cells and certain subsets of T cells, as well as on the surface of all intestinal intraepithelial lymphocytes (Mahoney 2015).

OX40 and OX40L

OX40 (TNFRSF4) is expressed on activated T cells, NK cells, NKT cells, and neutrophils. It functions as a secondary co-stimulatory immune checkpoint. Its ligand, OX40L is generally expressed during inflammation is found on APCs, including dendritic cells, B cells and macrophages. OX40L is expressed on activated APCs, but is not found on resting APCs. OX40 signaling helps effectuate response to T cell activation by supporting the survival and expansion of activated T cells, and the establishment of T cell memory.

GITR and GITRL

GITR (Glucocorticoid Induced TNFR Related Protein, TNFRSF18) is constitutively expressed on Tregs (T-regulatory cells). On resting CD4+ and CD8+ cells, GITL is upregulated about 24 hours after antigen activation and remains more highly expressed for several days thereafter. GITR is also expressed on various peripheral blood leukocytes and stimulated monocytes. Its ligand, GITRL is highly expressed on activated APCs and endothelial cells at sites of inflammation. GITR appears to play a role in promoting T effector cell activity by inducing proliferation and supporting survival in T cells, while also suppressing Treg activity (Mahoney 2015).

CD27 and CD70

CD27 is a co-stimulatory molecule that is expressed on numerous T lymphocytes, and certain memory B cells and NK cells. Its ligand, CD70, can be expressed on activated lymphocytes and is highly elevated in some tumors. Ligation of CD27 is involved in the activation and survival of NK cells, the maintenance of T cell effector functions, the development of T cell memory, support of germinal center formation and B cell maturation, and the production of high affinity IgG antibodies (Mahoney 2015).

CD30 and CD153

CD30 (TNFRSF8) is expressed by activated, but not resting, T and B cells while its ligand, CD153, is expressed on activated T cells. CD30/CD153 ligation mediates pleiotropic effects including cell proliferation, activation, differentiation and apoptosis. CD30 is overexpressed in various hematological malignancies, and is found in the leukocytes of patients with chronic inflammatory and autoimmune diseases including lupus erythematosus, asthma and rheumatoid arthritis.

CD40 and CD154

CD40 (TNFRSF5) is expressed on the surface of APCs, including B cells, dendritic cells, and macrophages as well as on the surface of endothelial cells, epithelial cells and a variety of tumor cells. Its ligand, CD154, is expressed on activated T cells, platelets, and several other cell types. The activation of CD40 is involved in germinal center formation, B cell development, and immunoglobulin isotype switching. Interaction of CD40 with CD154 is critical to co-stimulation and immune regulation. The importance of CD40 has been implicated in the pathology of multiple cardiovascular diseases, such as atherosclerosis and atherothrombosis.

4-1BB and 4-1BBL

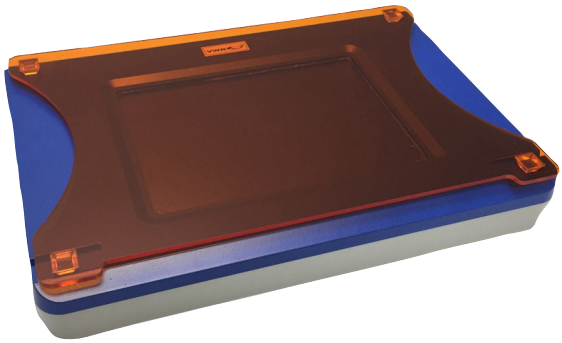
4-1BB (TNFRSF9) is mainly expressed on activated CD4+ and CD8+ T cells, activated B cells, dendritic cells, and natural killer cells; whereas, its ligand, 4-1BBL (TNFSF9), is mainly expressed on APCs, and activated B and T cells. Ligation of 4 1BB on T cells and natural killer cells induces cell activation, promotes survival, and enhances effector functions (Mahoney 2015).

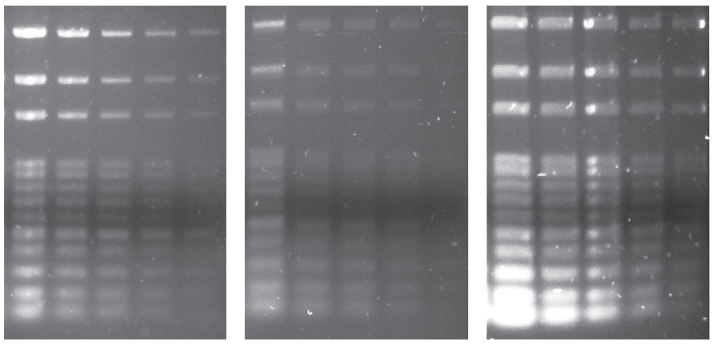
REFERENCES:

1. Ceeraz S, Nowak EC, Burns CM, Noelle RJ. 2014. Immune checkpoint receptors in regulating immune reactivity in rheumatic disease. Arthritis res & ther. 16(5):469.
2. Chen L, Flies DB. 2013. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nat Rev Immunol. 13(4):227-242.
3. Croft M. 2003. Co-stimulatory members of the TNFR family: keys to effective T-cell immunity. Nat Rev Immunol. 3(8):609-620.
4. Mahoney KM, Rennert PD, Freeman GJ. 2015. Combination cancer immunotherapy and new immunomodulatory targets. Nat Rev Drug Discov. 14(8):561-584.
5. Márquez-Rodas I, Cerezuela P, Soria A, Berrocal A, Riso A, González-Cao M, Martín-Algarra S. 2015. Immune checkpoint inhibitors: therapeutic advances in melanoma. Annals of Transl Med. 3(18):267-282.
6. Nguyen LT, Ohashi PS. 2015. Clinical blockade of PD1 and LAG3 - potential mechanisms of action. Nat Rev Immunol. 15(1):45-56.
7. Pardoll DM. 2012. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 12(4):252-264.
8. Sharpe AH. 2009. Mechanisms of costimulation. Immunol Rev. 229(1):5-11.
9. Topalian SL, Drake CG, Pardoll DM. 2015. Immune Checkpoint Blockade: A Common Denominator Approach to Cancer Therapy. Cancer Cell. 27(4):450-461.

Description	Size	Catalog Number	Unit
Human BDNF	100 µg	**10781-164**	**Each**
Human LIGHT	5 µg	**10774-182**	**Each**
Human PD L1	1 mg	**75999-602**	**Each**
Human 4-1BB Ligand	1 mg	**10774-208**	**Each**
Human sCD 40 Ligand	100 µg	**10780-644**	**Each**
Human OX40 Ligand	1 mg	**10770-896**	**Each**
Murine LIGHT	1 mg	**10770-936**	**Each**
Murine sCD 40 Ligand	1 mg	**10780-742**	**Each**

A safer way to visualize your DNA





Comparison of EZ-Vision® Blue Light DNA Dye to GelGreen™ and SYBR® Green I Two-fold dilution series of identical DNA fragments were electrophoresed in 1% agarose gels. The gels were then stained with EZ-Vision Blue Light (left panel), GelGreen (middle panel) and SYBR Green I (right panel). DNA fragments were visualized using a blue light transilluminator and SYBR Green filter.

VWR® BLUE LIGHT TRANSILLUMINATOR

- Blue wavelength does not damage DNA
- Uniform illuminated surface
- 465 nm wavelength, ideal for most fluorescent DNA stains
- Amber cover is removable and can be set to an angle

VWR®'s Blue Light Transilluminator provides a safe and cost-effective alternative to UV transilluminators for viewing gels stained with dyes excited in the blue light spectrum. Blue light excitation does not damage DNA samples and these dyes can be safe alternatives to hazardous ethidium bromide. The evenly illuminated viewing area can accomodate gels up to 12 x 17 cm, and the scratch-resistant glass surface allows cutting out bands.

Electrical	Cat. No.
100 to 240VAC (Includes US type plug)	**76151-834**

EZ-VISION® BLUE LIGHT PERFOMANCE VERSUS COMPETING BLUE LIGHT STAINS

BLUE LIGHT VISUALIZATION

Fluorescent dye visualizes DNA bands using blue light excitation or standard UV transilluminator.

LESS DAMAGE TO DNA

Use with blue light excitation (410–510nm) and a green emission filter (500–600 nm) to eliminate UV-induced DNA damage and improve downstream cloning efficiency.

SAFER TO USE

Formula is both non-toxic and non-mutagenic and eliminates the need to use ethidium bromide and UV light.

FLEXIBILITY AND COMPATIBILITY

Use as an in-gel stain for immediate post-electrophoresis results or as an easy 30 minute post-stain. Compatible with standard downstream applications, including gel extraction, cloning and sequencing.

Description	Size, µL	Cat. No.
EZ-Vision Blue Light Dye, 10,000X	500	**10791-798**

IgGy Antibody Selector

Search. Select. Simple

VISIT VWR.COM/ANTIBODY

Using the IgGy Antibody Selector makes searching for antibodies easier. VWR has brought together a multitude of antibody suppliers and manufacturers with hundreds of thousands of antibodies to meet your specific application needs. With VWR, IgGy offers:

- More than 350,000 antibodies
- Brands you know and trust
- Wide range of conjugations
- Choices from multiple suppliers
- Resource for all application areas

ABGENT envision proteomics
Abnova
AdipoGen Connecting Immunology to Metabolism
BioCell
biogems A PeproTech Brand
BioLegend The path to legendary discovery
Bioss Antibodies
Biotium
BioVision BioVision Incorporated Innovation. Flexibility. Affordability
BOSTER antibody and ELISA experts
Diagnostic BioSystems
Enzo
Electron Microscopy Sciences
Fitzgerald
GeneTex
ImmunoReagents Inc.
JACKSON
NOVUS BIOLOGICALS
PEPROTECH OUR SUPPORT, YOUR DISCOVERY
ProSci Incorporated
proteintech antibodies
PromoCell
ROCKLAND antibodies & assays
SB Sino Biological
SouthernBiotech
TONBO biosciences Equivalent Performance, Exceptional Value

T cell receptor characterization of PBMCs using the CytoFLEX

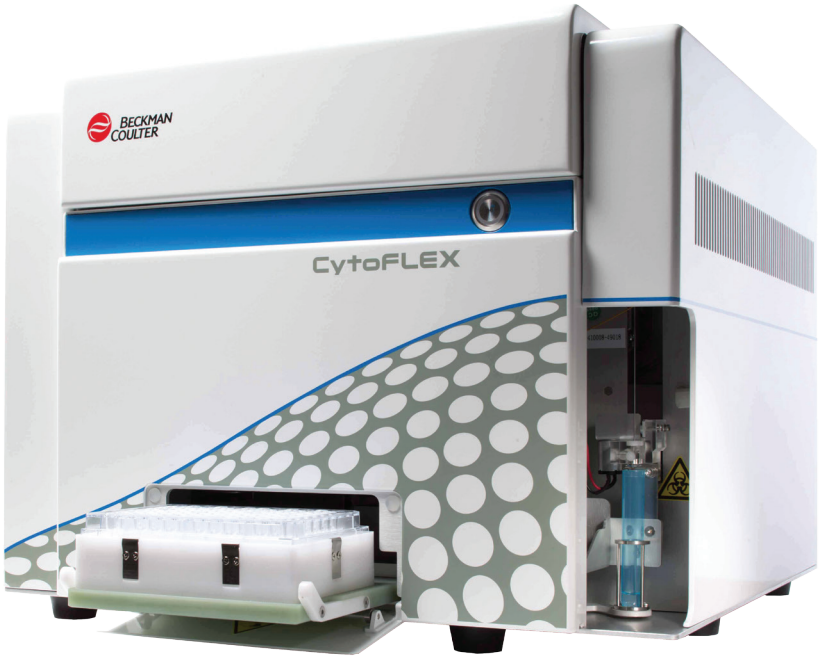
By Michael McPherson, Karen Carr, Si-Han Hai, Beckman Coulter

T cells play a pivotal role in the function and regulation of the immune system. Mature T cells express both CD3 and a T Cell Receptor (TCR). They can further be divided into two subpopulations based on their type of TCR: αβ T cells (95% of the T cell repertoire) and γδ T cells (5% of the T cell repertoire).

Visit **vwr.com/cytoflex** for more information

TCR αβ T cells are then differentiated into CD4 T cells or CD8 T cells based on their affinity recognition of antigens bound to MHC Class II or MHC Class I, respectively. The largest subset of γδ T cells express the Vδ2 TCR complex. A smaller subset of γδ T cells express the Vδ1 TCR complex.

The study of TCR γδ Vδ2 T cells and TCR γδ Vδ1 T cells is important in feto-maternal[1] and allograft tolerance[2]. The understanding of the many T cell subpopulations is important for a variety of normal and pathological studies.



DuraClone IM panels are unitized, dry format reagent cocktails that are room temperature stable. Tube formulations were based on the ONE Study as well as design input from expert flow Cytometry labs for use in clinical research studies. Here, we look at the DuraClone IM TCRs tube with normal PBMCs on the CytoFLEX.

METHODS

Peripheral Blood Mononuclear Cell (PBMC) isolation

10mL of whole blood was diluted to 21mL with 1X PBS + 3% FCS; 6mL of Ficoll was added to three, 15mL conical vials; 7mLs of diluted blood were added to the Ficoll by addition with a 10mL pipet at a very steep angle. Diluted blood was then slowly added to over-lay onto the Ficoll. Cells were spun at 1500 RPM for 30 minutes in a swinging bucket centrifuge with the brake turned off. Supernatant was aspirated and samples were pooled and washed in PBS + 5% FCS. Cell pellets were resuspended and counted using the ViCell and diluted to a final concentration of 1 x 10^6 cells/100µL per tube for staining.

DuraClone Staining Procedure

One million PBMCs were added to a DuraClone IM TCRs tube and then vortexed for 5 seconds. After 20 minutes incubation at room temperature in the dark, the PBMCs were washed with 3mL of PBS and centrifuged at 400g for 5 minutes. The PBMCs were resuspended in 250µL of PBS and acquired on the CytoFLEX.

RESULTS

Rapid TCR Subsetting with the DuraClone IM TCRs Tube

Staining of normal PBMCs with the DuraClone IM TCRs tube depicted here suggests that T cell subsets can be rapidly determined with the CytoFLEX. After approximately 30 minutes,

REAGENT DETAILS

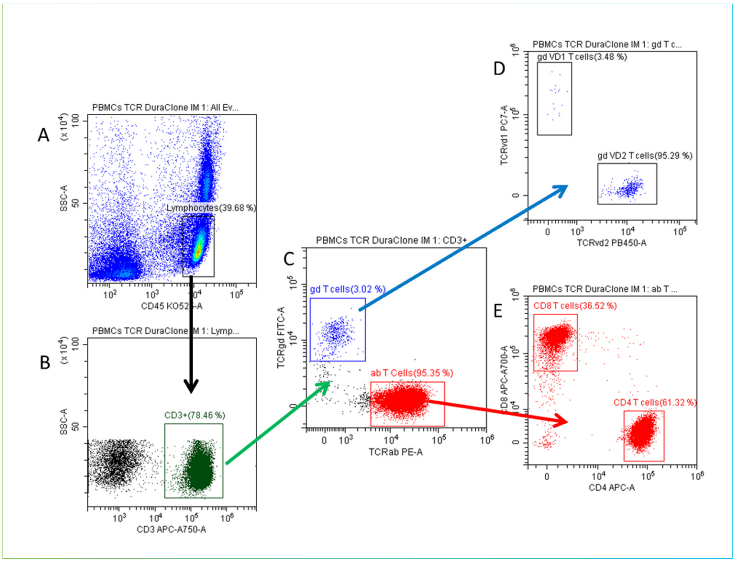


FIGURE 1: **Rapid T cell receptor subsetting with the DuraClone IM TCRs.** Tube PBMCs were gated on lymphocytes by CD45 staining (A). CD3+ lymphocytes (B) were then gated based on expression of TCR γδ or TCR αβ (C). Of the γδT cells, expression of Vδ1 and Vδ2 was characterized (D). Of the αβ T cells, CD8 and CD4 expression is characterized (E).

PBMCs were accurately characterized into their appropriate compartments. Using the DuraClone IM TCRs tube is a viable alternative to laborious staining procedures.

REFERENCES

1. Mincheva-Nilsson L. 2003. Pregnancy and gamma/delta T cells: taking on the hard questions. Reprod Biol Endocrinol. 2(1):120. Dec 2; 1:120.
2. Yu X, Liu Z, Wang Y, Zhang M, Sun Y, Su H, Jin L, Wang F, Shi M. 2013. Characteristics of Vδ1 (+) and Vδ2 (+) γδ T cell subsets in acute liver allograft rejection. Transpl Immunol. 29(1- 4):118-22.

NOTES

The results demonstrated in this application sheet represent those generated on the Beckman Coulter CytoFLEX Flow Cytometer. As differences exist in the performance between analyzers, the authors cannot guarantee a similar appearance with the use of other flow cytometers.

RESULTS

Laser	405 nm					488 nm					638 nm		
Fluor	Krome Orange	Pacific Blue	V610	V660	V780	FITC	PE	ECD	PC5	PC7	APC	AF700	APC AF750
Marker	CD45	TCR Vδ2				TCR γδ	TCR αβ	HLA-DR		TCR Vδ1	CD4	CD8	CD3
Clone	J.33	IMMU 389				IMMU510	IP26A	Immu-357		R9.12	13B8.2	B9.11	UCHT-1

Simple, rapid, and efficient labeling of antibodies and proteins for multicolor immunofluorescence microscopy and flow cytometry

By Saloni Pasta and Lori Roberts; Biotium, Inc., Freemont, CA

Mix-N-Stain™ LABELING

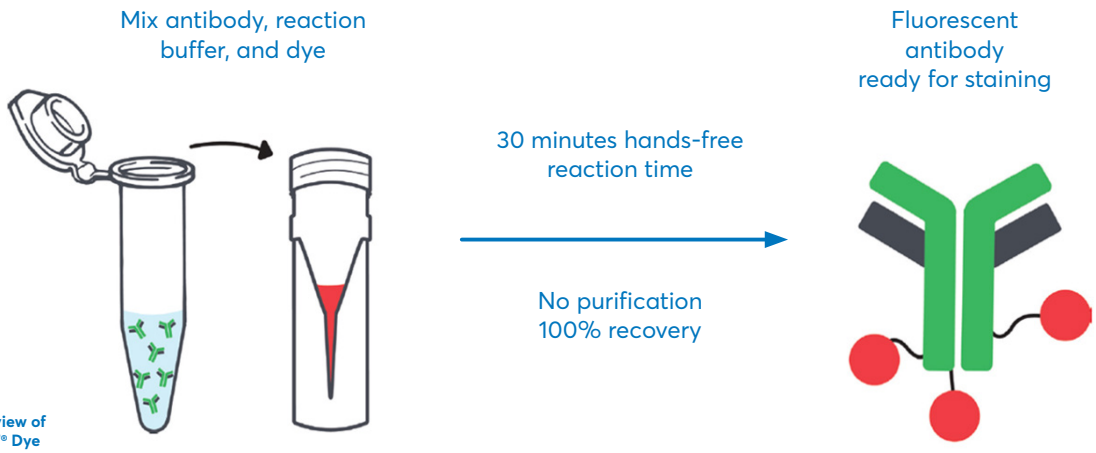


FIGURE 1: **Overview of Mix-n-Stain™ CF® Dye Antibody Labeling.** Simple, efficient antibody labeling protocol.

Proteins rarely operate in isolation. There is growing interest in simultaneously analyzing multiple protein targets for a more complete understanding of underlying cellular physiology or disease states. Multicolor immunostaining analysis is a powerful tool in cellular analysis to determine both the presence of multiple protein targets on a particular cell or tissue type, and by cytometry, to quantitate the amount of the protein target in each cell. Multicolor staining using a combination of fluorescently tagged primary and secondary antibodies is driving the need for efficient and simple-to-use antibody labeling technologies.

Mix-n-Stain™ Antibody Labeling Kits from Biotium dramatically simplify the process of fluorescently labeling antibodies for fluorescence microscopy, flow cytometry, near-infrared Western blotting and other life science applications. Simply mix your antibody with a CF® dye of your choice in the provided reaction buffer, a step that takes less than 30 seconds of hands-on time (Figure 1). There is no need to calculate how much dye you should use, just mix your antibody with the entire amount of dye provided to optimally label your antibody. After a 30-minute incubation, and without the need for subsequent purification, the fluorescent antibody conjugate is ready to use

(Figure 2). Choose from a wide range of CF® dye colors, biotin, or other haptens for multicolor flexibility.

Mix-n-Stain™ labeling can tolerate the presence of common stabilizers, such as sodium azide, and low concentrations of Tris, BSA, and glycerol. An ultrafiltration centrifuge vial provided in the kit can be used to quickly clean up antibodies containing incompatible small molecules, such as glycine and high concentrations of Tris or glycerol. In addition, you can label your antibody in the presence of excess stabilizer protein such as BSA or gelatin by selecting a kit size based on the amount of total protein in the antibody solution following our new modified labeling protocol. The modified protocol also allows you to label less than 5µg antibody.

Mix-n-Stain™ Antibody Labeling Kits contain revolutionary labeling reagents designed to facilitate multicolor fluorescent detection and offer significant benefits over other commercially available antibody labeling kits. A major advantage is our superior CF® dyes. The CF® dyes rival or exceed the brightness and photostability of Cy® dyes, Alexa Fluor®, DyLight® and other dyes. Additionally, the higher water solubility of the CF® dyes allows a higher degree of labeling on the conjugates. The performance and brightness of Mix-n-Stain™ labeled conjugates is therefore superior and rivals that of purified antibody conjugates from other leading suppliers (Figure 2), such as the Lightning Link™ kits which use DyLight® dyes (Figure 3). The Mix-n-Stain™ kits are also available in wider range of sizes to label from low µg to mg quantities of antibody for greater flexibility. In contrast to the kits that use antibody fragments for physically labeling primary antibodies, Mix-n-Stain™ labeling is covalent. Mix-n-Stain™ labeling is therefore not species-specific, and no fixation step is required after staining.

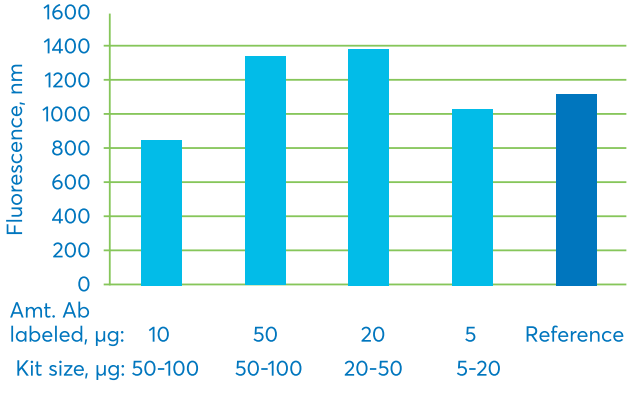


FIGURE 2: Flow cytometry analysis of Jurkat cells stained with various CF®633-labeled mouse anti-human CD3 antibodies. The fluorescent antibody conjugates were prepared from different amounts of the unlabeled antibody and matching labeling kits as detailed in the figure. For reference, cells were also stained with commercial mouse anti-human CD3 pre-labeled with Alexa Fluor® 647 and analyzed on a BD FACSCalibur™ in the FL4 channel.

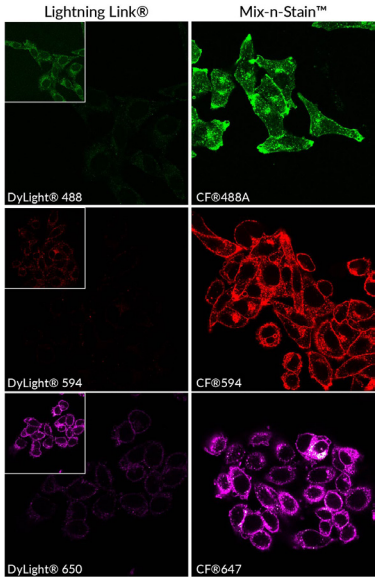


FIGURE 3: Comparison of Lightning Link® labeling with DyLight® 488 and Mix-n-Stain™ labeling with CF®488A. Mouse anti-transferrin receptor antibody from (endosome and plasma membrane marker) was labeled using Lightning-Link® Rapid DyLight® Conjugation Kits from Novus Biologicals (left) or Mix-n-Stain™ CF® Dye Antibody Labeling Kits (right) according to manufacturers' instructions. The CF® dye conjugates show higher signal and more specific staining compared the DyLight® conjugates when imaged using the same settings, due to the superior brightness of CF® dyes. The insets show the same field of view imaged with a higher gain setting.

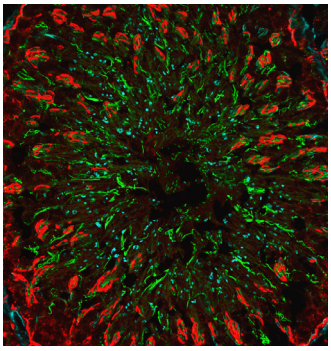


FIGURE 4: Combined direct and indirect immunofluorescence staining. Rat testis are stained with mouse anti-tubulin and CF®488A goat anti-mouse (min x rat) (microtubules, green), CF®555 Mix-n-Stain™ labeled mouse anti-ZO1 (tight junctions, red) and CF®640R phalloidin (actin filaments, cyan).

In addition, Mix-n-Stain™ conjugates are stable for at least 6 months at 4°C in the antibody storage buffer provided in the kit, while kits using antibody fragments antibody complexes must be used within 30 minutes.

These novel dyes and labeling kits allow rapid preparation of fluorescently labeled primary antibodies for multicolor immunofluorescence applications (Figure 4).

Biotium also offers Mix-n-Stain™ Kits for labeling antibodies with enzymes (HRP, AP, or GOx) or fluorescent proteins for flow cytometry (PE, APC, PerCP, and tandem dyes). Labeling with these kits takes about 2 hours to complete, with minimal hands-on time and no purification after labeling.

Description*	Cat. No.	Unit
CF®405S Mix-n-Stain™ Antibody Labeling Kit	89171-578	Each
CF®488A Mix-n-Stain™ Antibody Labeling Kit	89171-582	Each
CF®555 Mix-n-Stain™ Antibody Labeling Kit	89171-584	Each
CF®594 Mix-n-Stain™ Antibody Labeling Kit	89171-588	Each
CF®647 Mix-n-Stain™ Antibody Labeling Kit	89171-594	Each
CF®660R Mix-n-Stain™ Antibody Labeling Kit	89171-598	Each
CF®680R Mix-n-Stain™ Antibody Labeling Kit	89171-602	Each
CF®770 Mix-n-Stain™ Antibody Labeling Kit	89171-606	Each

* The Mix-n-Stain kits are available with over 20 CF dye options (from UV to near-IR), biotin, and other haptens. The kits offered here are enough to label 5–20µg of antibody. Please contact your VWR Life Science Specialist for additional dye options or sizes.

ZYMO RESEARCH
The Beauty of Science is to Make Things Simple

RNA directly from samples in TRIzol® — in just 7 minutes!

DIRECT-ZOL™ RNA KITS

- **Easy-handling:** Bypass chloroform, phase separation, precipitation and post-extraction clean-up steps
- **NGS-ready:** Ultra-pure RNA without phenol carryover and no DNA contamination (DNase I included)
- **Non-biased:** Complete RNA recovery without miRNA loss

The Direct-zol™ RNA Kits provide a streamlined method for the purification of high-quality RNA directly from samples in TRIzol®. The procedure is simple. Simply apply a sample prepared in TRIzol® directly to the Zymo-Spin™ Column and then bind, wash, and elute the RNA. No phase separation, precipitation, or post-purification steps are necessary.

US ONLY

Description	RNA Capacity	Rxn Size	Cat. No.	Unit
Direct-zol™ RNA Microprep	10 µg	50	**76020-456**	**Each**
Direct-zol™ RNA Microprep	10 µg	200	**76211-324**	**Each**
Direct-zol™ RNA Miniprep Plus	100 µg	50	**76020-110**	**Each**
Direct-zol™ RNA Miniprep Plus	100 µg	200	**76211-328**	**Each**
Direct-zol™-96 RNA Kit	10 µg	2 x 96	**76020-452**	**Each**
Direct-zol™-96 RNA Kit	10 µg	4 x 96	**76211-344**	**Each**
Direct-zol™-96 MagBead RNA	10 µg	2 x 96	**76211-332**	**Each**
Direct-zol™-96 MagBead RNA	10 µg	4 x 96	**76211-348**	**Each**

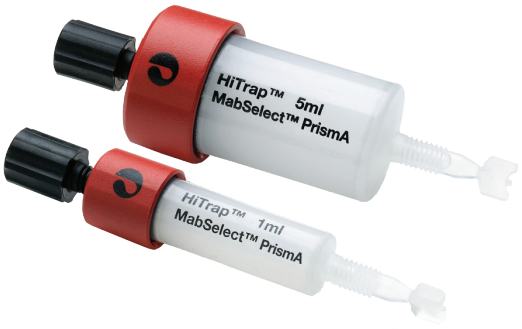
At the time of publication, these products were not available in Canada. Please contact your VWR Life Science Specialist to learn about similar options available in your region.

Enhanced alkaline stability and dynamic binding capacity

Distributor
GE Healthcare

HITRAP™ MABSELECT™ PRISMA AND HISCREEN™ MABSELECT PRISMA COLUMNS

- High dynamic binding capacity compared with other protein A resins for improved antibody recovery
- Excellent alkaline stability for efficient cleaning between runs using 0.5–1.0 M NaOH
- Formats are well-suited for preparative purifications, as well as for screening and optimization of purification conditions



MabSelect PrismA is a next-generation protein A affinity chromatography resin that offers significantly enhanced alkaline stability and capacity for improved performance in antibody purifications.

Size	Cat. No.	Unit
HiTrap MabSelect PrismA Columns		
1 x 1 mL	**76237-726**	**Each**
5 x 1 mL	**76237-728**	**Each**
1 x 5 mL	**76237-730**	**Each**
5 x 5 mL	**76235-124**	**Each**

Size	Cat. No.	Unit
HiScreen MabSelect PrismA Column		
4.7 mL	**76237-722**	**Each**
MabSelect PrismA Chromatography Resin		
25 mL	**76237-724**	**Each**
200 mL	**76237-732**	**Each**

Increasing assay efficiency with four-color detection

By Joy DeTorres, Product Manager, Azure

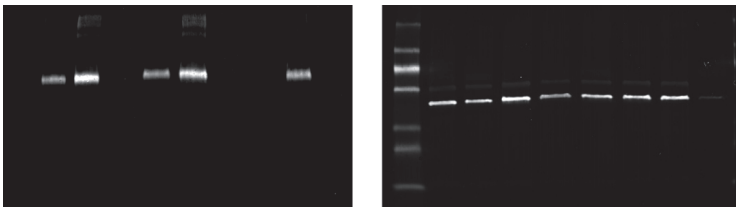
The field of Western blot multiplexing – the ability to probe for multiple proteins on a single blot simultaneously – is developing rapidly. Chemiluminescent assays allowed for the detection of a single protein followed by multiple rounds of time consuming stripping and re-probing, with associated potential loss of protein and corresponding signal.

Early Western blot multiplex imaging systems allowed for the imaging of two spectrally distinct fluorophores on a single blot. Researchers rapidly used this technique to assay for loading controls and proteins of interest on a single blot, to compare two distinct proteins, and to devise many other methodologies which are discussed in other application notes.

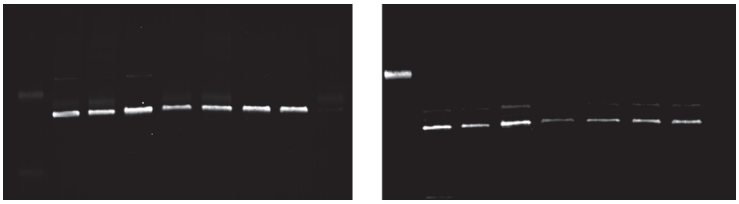
We have previously described an improvement on this two-channel methodology by imaging three proteins simultaneously by combining the fluorescent and nearinfrared (NIR) imaging capabilities offered by the Azure Biosystems c600 digital imager.

In this note we discuss a further improvement – four-color Western blot multiplexing using the Azure Biosystems Sapphire™ Biomolecular Imager. The ability to simultaneously image four colors at one time greatly increases Western blot efficiency and the ability to make meaningful quantitative comparisons. Four-color Western blotting is made possible through the

A. 490 – transferrin (blue) B. 55 – tubulin (green)



C. 700 – actin (red) D. 800 – GAPDH (gray)



E.

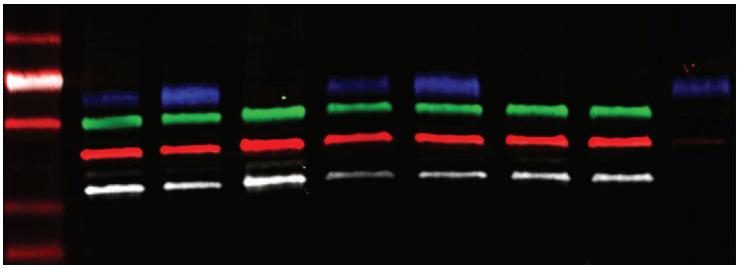


FIGURE 1: **Digital images of 4-color Western blot, captured using Azure Biosystems Sapphire™ Biomolecular Imager.** Transferrin (a), tubulin (b), actin (c) and GAPHD (d) were probed on a single blot using distinct fluorescent and near-infrared targeting antibodies. Images were captured using the Azure Biosystems Sapphire™ Biomolecular Imager at the specified wavelengths and merged into the four-color multiplex image (e). Sensitive and specific detection of all four proteins was possible with no evidence of background auto-fluorescence or bleed between channels.

use of four spectrally distinct fluorophores and the Sapphire's selective laser based excitation and sensitive PhotoMultiplier Tube (PMT) and Avalanche PhotoDiode (APD) detection systems.

MATERIALS AND METHODS

Run and Transfer Gel

Samples of 1.25 to 5 µg of HeLa cell lysate, some spiked with varying amounts of transferrin were electrophoresed on a Tris-Glycine gel. After electrophoresis and separation, proteins were transferred to a low fluorescence PVDF membrane using Azure Transfer Buffer.

Four-color Western Blotting

Following transfer, the membrane was blocked for 30 minutes with Azure Fluorescent Blot Blocking Buffer then probed with rat anti-tubulin (green), rabbit anti-beta actin (red), and chicken anti-GAPDH (gray) primary antibodies; and anti-transferrin (Blue) which had previously been labelled with AzureSpectra 490 dye using the AzureSpectra Labeling Kit.

Blots were rinsed and washed with Azure Fluorescent Blot Washing Buffer before being incubated with AzureSpectra labeled secondary antibodies – goat anti-rat 550 (green), goat anti-rabbit 700 (red), and goat anti-chicken 800 (gray). After incubation, the blot was washed as before in Azure Florescent Blot Washing Buffer followed by a final rinse in PBS.

Image Four-color Western Blot

After rinsing in PBS, the blot was allowed to dry before imaging on the Azure Biosystems Sapphire™ Biomolecular Imager.

RESULTS AND CONCLUSIONS

In this note a single Western blot was probed for four proteins simultaneously. HeLa cell samples, or HeLa cell samples spiked with transferrin were probed with tubulin (green), beta-actin (red) and GAPDH (gray) antibodies followed by isotype appropriate secondary antibodies; as well as transferrin (blue) antibody previously conjugated with AzureSpectra 490 dye for direct analysis.

The Western blot was imaged using the Azure Biosystems Sapphire™ Biomolecular Scanner and Figure 1 shows the grayscale image captured at each wavelength for transferrin (a), tubulin (b), beta-actin (c) and GAPDH (d); together with a merged, colorized image (e). Together the images demonstrate the high level of sensitivity, specificity and lack of background signal it is possible to achieve using this methodology which allows for rapid and accurate quantitative analysis.

The ability to image four proteins on a single blot greatly increases the efficiency of Western blotting, saving time and precious samples and allows for better quantitative analysis. The methodology also allows for the use of novel assays such as investigations of protein:protein interactions or on-blot total vs phosphorylated protein assays.

Step	Product	Part Number
	4-15% Tris-Glycine gel	N/A
Electrophoresis & transfer	PVDF Membrane	**10147-300**
	Azure Transfer Buffer	**10147-348**
Blocking & antibody labelling	AzureSpectra Fluorescent Blot Blocking Buffer	**75794-864**
	AzureSpectra Labeling Kit – 490	**75794-836**
	Primary Antibodies	Per protein of interest
	AzureSpectra Goat-anti-rat-550	**75794-882**
	AzureSpectra Goat-anti-rabbit-700	**10147-350**
	AzureSpectra Goat-anti-chicken-800	**10147-368**
Probe blot	PBS	N/A

TABLE 1: Material and product numbers.

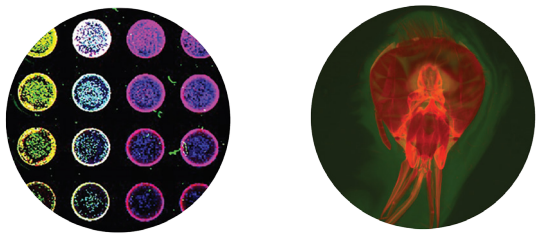
DNA stains and equipment that are safe for you and your samples

SAPPHIRE BIOMOLECULAR IMAGER

MORE IMAGING MODALITIES, RESOLUTION, SENSITIVITY, APPLICATIONS, VALUE

With outstanding performance for fluorescence, chemiluminescence, and phosphor imaging, the Sapphire Biomolecular Imager expands what you can accomplish with a single image scanning instrument. Experience high resolution (10 micron) and large field of view (25 x 25cm) in a modular system that allows you to configure the instrument to support only the applications you need.

- Fluorescent western blot
- 2D-DIGE
- Fluorescent protein native gel
- Reporter gene assay
- Nucleic acid gel documentation
- ELISA in-gel western
- In-cell westerns
- Protein gel documentation
- Protein array
- In-cell or on-cell ELISA
- Densitometry
- Chemiluminescent array
- Chemiluminescent western blot
- Quantitative western blot
- Electrophoretic mobility assay
- Phosphor imaging
- Tissue section imaging



Contact your Life Science Specialist for special pricing.

Description	Includes	Cat. No.
Sapphire Biomolecular Imager, NIR	Laser excitation wavelengths at 658 nm and 785 nm, Sapphire mat, USB connector, and power cable	76317-604
Sapphire Biomolecular Imager, RGB	Laser excitation wavelengths at 488 nm, 520 nm and 658 nm, Sapphire mat, USB connector, and power cable	76317-606
Sapphire Biomolecular Imager, RGBNIR	Laser excitation wavelengths at 488 nm, 520 nm, 658 nm and 785 nm, Sapphire mat, USB connector, and power cable	76317-608
Sapphire Biomolecular Imager, PI	Laser excitation wavelength at 658 nm, Sapphire mat, USB connector, and power cable	76317-610
Accessories		
Chemiluminescence module	Add chemiluminescence detection and white light imaging to Sapphire Biomolecular Imager	76291-948
Phosphor Imaging module	Add phosphor imaging to Sapphire Biomolecular Imager	76291-950
Q-Module, 520 Laser module	Add 520 nm laser and detector to Sapphire Biomolecular Imager, NIR	76291-952

VWR bioMarke™
VOLUME 5 ISSUE 1

VWR LIT LIBRARY
c/o Archway
20770 Westwood Drive
Strongsville, OH 44149

PRSRT STD
US POSTAGE
PAID PERMIT 293
MIDLAND MI

ATTN: POST OFFICE: ELECTRONIC SERVICE REQUESTED

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

0519 30M Lit. No. 930833

ENVIRO/TECH INK
Enviro/Tech is a registered trademark.

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

Setting science in motion to create a better world

avantor™

From breakthrough discovery to agile delivery, we offer an extensive portfolio of mission-critical products, services, and solutions. We are a trusted global partner to customers in the life sciences, advanced technologies, and applied materials industries.

vwr™ part of avantor

VWR, part of Avantor provides an integrated, seamless purchasing experience optimized for your success.

Learn how the new Avantor is moving forward at avantorsciences.com

PB18015-EN