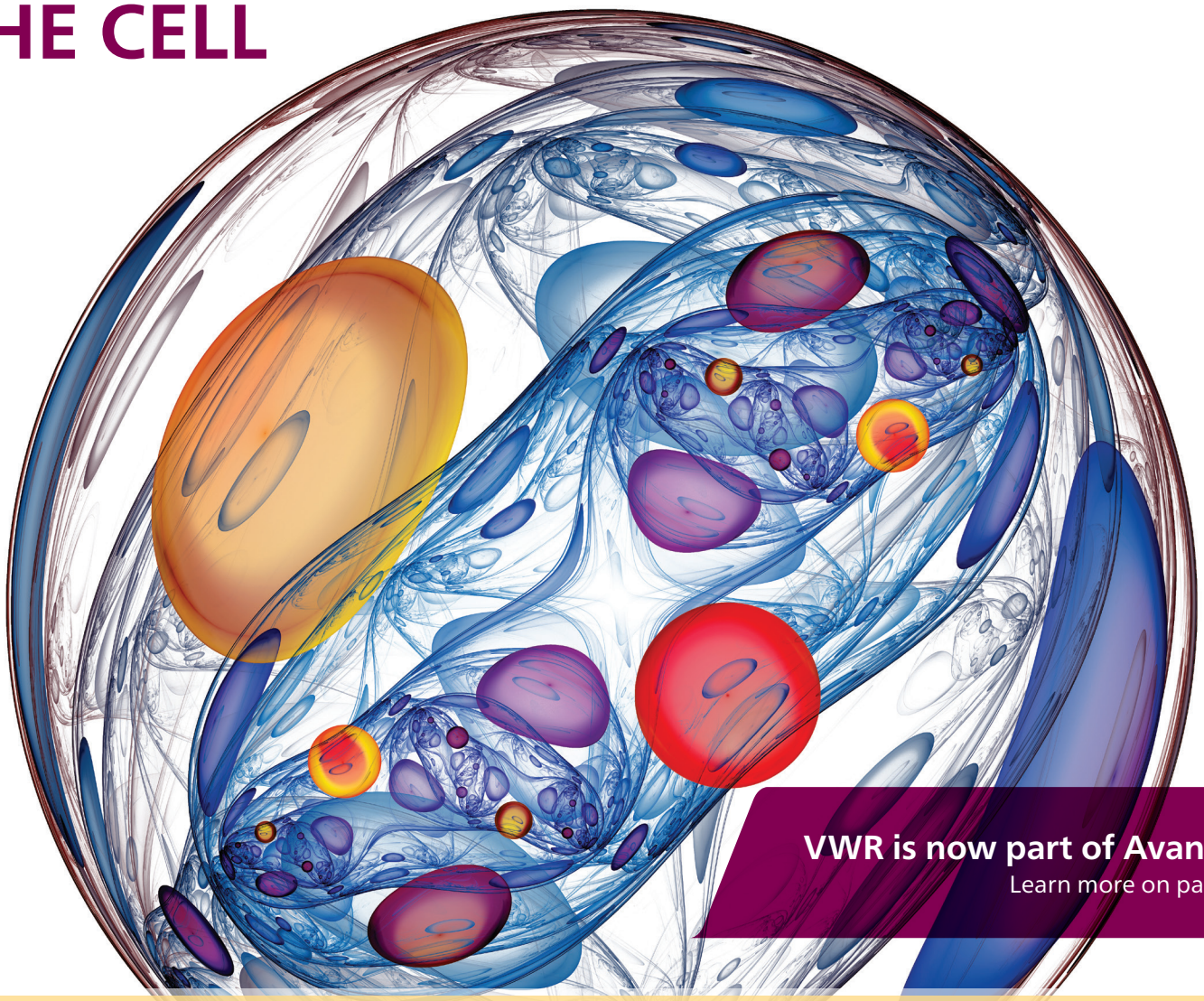
VWR®
We Enable Science

Volume 4, Issue 1

VWR bioMarke™

ADVANCING THE CELL



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ADVANCING THE CELL…

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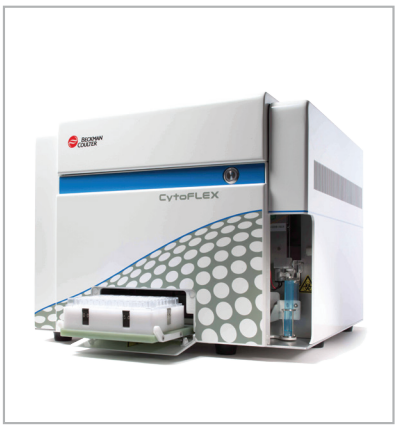

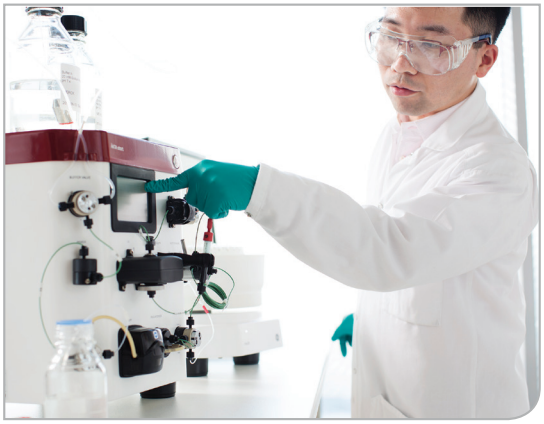

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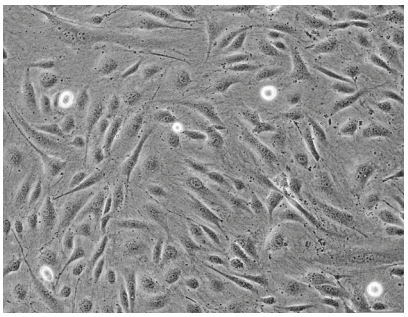
New Endothelial Progenitor Cells

StemBioSys®

StemBioSys® CELLvo™ Human Cord Blood Progenitor Cells (hCB-EPCs) for Basic and Translational Research



- CELLvo hCB-EPCs form secondary and tertiary colonies
- CELLvo hCB-EPCs have greater intrinsic vessel formation capacity
- CELLvo hCB-EPCs exhibit a pro-angiogenic cell profile

CELLvo Human Cord Blood - Endothelial Progenitor Cells (hCB-EPC) are derived from a single donor and cryopreserved. hCB-EPCs can be ordered as a single vial of cells or as a kit that includes StemBioSys CELLvo Matrix plates for optimal growth and proliferation.

Description	Cat. No.	Unit
Human Cord Blood Endothelial Progenitor Cells from a Single Donor, Cryopreserved at P4, >500,000 Cells	76169-452	Each
Kit Contains hCB-EPCs Plus a Sleeve of 5 CELLvo Matrix 6-Well Plates	76175-646	Each
Kit Contains hCB-EPCs Plus a Sleeve of 5 CELLvo Matrix T75 Flasks	76175-648	Each
Kit Contains hCB-EPCs Plus a Sleeve of 5 CELLvo Matrix T150 Flasks	76175-650	Each
CELLvo Matrix Plates and Flasks, Irradiated		
CELLvo Matrix 6-Well Culture Plate	75853-938	Pack of 5
CELLvo Matrix T75 Culture Flask	75853-856	Pack of 5
CELLvo Matrix T150 Culture Flask	75853-858	Pack of 5

VWR COLLECTION

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VWR® LIFE SCIENCE

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- **RPMI:** Available with or without L-glutamine
- **Balanced Salt Solutions and Reagents:** Dulbecco's Phosphate Buffered Saline (DPBS), HBSS, Trypsin, and L-Glutamine

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

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

Ingenio® Electroporation Solution and Kits

Highly Efficient and Cost Effective Electroporation Using Any Instrument

- **High Efficiency Electroporation —** Deliver to hard-to-transfect cell lines and primary cells
- **Compatible with Conventional Electroporation Devices —** Use your existing system including Lonza Amaxa®, Bio-Rad®, or Harvard BTX®
- **Cost Effective —** Ingenio Electroporation Solution is available as a stand-alone solution or as part of a complete kit with cuvettes and cell droppers

Ingenio electroporation solution and kits provide a universal, high efficiency, low toxicity solution for electroporation of DNA, small RNA (siRNA, miRNA, CRISPR gRNA), or Cas9 RNP into hard-to-transfect cell types. Electroporation using Ingenio affords increased gene expression in several different cell types with minimal toxicity. Ingenio is also compatible with multiple conventional electroporation instruments including Lonza Amaxa Nucleofector®, Bio-Rad Gene Pulser®, and Harvard-BTX.

Free samples are available at **vwr.com/mirusbio.**

US ONLY

Description	Number of Reactions	Cat. No.	Unit
Ingenio Electroporation Solution	25	10766-836	Each
Ingenio Electroporation Solution	50	10766-842	Each
Ingenio Electroporation Solution	100	10766-848	Each
Ingenio Electroporation Kit with 0.2 cm Cuvettes for Lonza Amaxa Nucleofector II/2b	25	10766-838	Each
Ingenio Electroporation Kit with 0.2 cm Cuvettes for Lonza Amaxa Nucleofector II/2b	50	10766-844	Each
Ingenio Electroporation Kit with 0.2 cm Cuvettes for Lonza Amaxa Nucleofector II/2b	100	10766-850	Each
Ingenio Electroporation Kit with 0.4 cm Cuvettes for other conventional electroporators, such as Bio-Rad and Harvard-BTX	25	10766-840	Each
Ingenio Electroporation Kit with 0.4 cm Cuvettes for other conventional electroporators, such as Bio-Rad and Harvard-BTX	50	10766-846	Each
Ingenio Electroporation Kit with 0.4 cm Cuvettes for other conventional electroporators, such as Bio-Rad and Harvard-BTX	100	10766-990	Each
Accessories			
Ingenio Cell Droppers	—	10767-002	Pk. 50
Ingenio® Cuvettes, 0.2 cm	—	10766-992	Pk. 25
Ingenio® Cuvettes, 0.2 cm	—	10766-994	Pk. 50
Ingenio® Cuvettes, 0.4 cm	—	10766-998	Pk. 50

These specific products are not available in Canada. Please contact your VWR Sales Rep to learn about similar options available in your region.

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Water for Cell Culture and Molecular Biology

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Purified and Nuclease-Free

- Low endotoxin
- Purified and nuclease-free

Ideal for use in the preparation of cell culture media and reagents. For further manufacturing or research use only.

Description	Size	Cat. No.	Unit
Sterile Water, Nuclease-free	100 mL	97062-790	Each
Sterile Water, Nuclease-free	500 mL	97062-794	Each
Sterile Water, Nuclease-free	1 L	97062-792	Each
Molecular Biology Grade Water	500 mL	VWRL0201-0500	Each
Molecular Biology Grade Water	1 L	VWRL0201-1000	Each
Tissue Culture Grade Water	500 mL	VWRL0200-0500	Each
Tissue Culture Grade Water	1 L	VWRL0200-1000	Each

CELLSTAR® Cell Culture Vessels with Cell-Repellent Surface

Introduction

For most applications in cell culture, the properties of the vessels to be used are optimised to enhance conditions for cell attachment. With platforms made of polystyrene, this is achieved by using a physical method to treat the surface of the vessels. This treatment leads to the incorporation of polar carboxyl and hydroxyl groups to the hydrophobic polystyrene surface resulting in improved and very regular cell attachment. For fastidious cell lines or applications that will stress the cells, protein coated surfaces or advanced non-biological surfaces are applied.

Nevertheless, for some applications a surface that prevents cell attachment is required. These applications include:

- Spheroid culture
- Aggregation of stem cells
- Suspension culture of semi-adherent and adherent cell lines

The CELLSTAR cell-repellent surface from Greiner Bio-One fulfills these demands. Achieved through an innovative chemical surface modification, Greiner Bio-One's cell-repellent surface is an ideal substrate for cell culture as it does not degrade or 'leach out' under standard cell culture conditions.

All cell culture vessels with a cell-repellent surface are sterilized by irradiation (SAL of 10^{-3}). They are quality controlled for the absence of detectable endotoxins, DNase/RNase and human DNA and show no cytotoxic effects. Evaluation of cytotoxicity is done in accordance to EN ISO 10993-5 with mammalian cell lines.

To control the performance of the surface of cell-repellent cell culture vessels, attachment of cells is compared to the standard tissue culture CELLSTAR® surface. $CaCO_2$ cells are seeded at a density of 18,000 cells/cm^2 and incubated for 24 hours at 37°C in an incubator with a 5% CO_2 environment. After 24 hours of incubation, the medium is removed, the vessels are washed with PBS and EtOH is added to fix adherent cells, if present. The sample vessels are then examined and compared under a microscope with 10-fold magnification (Fig. 1).

Application	Tested Cell Lines
Cell Adhesion	Vero MDCKII Alveolar Macrophages (Rat) $CaCO_2$ Jurkat CHO SK-N-MC STO-DA
Stem Cell Aggregate Formation	mES-D3 human iPSCs
Spheroid Culture	HeLa HepG2 LNCaP HEK-239

Table 1: Tested cell lines

Inhibition of Cell Attachment of Semi-Adherent and Adherent Cell Lines in Vessels with Cell-Repellent Surface

For the culture of suspension cells, surfaces of a strong hydrophobic nature are

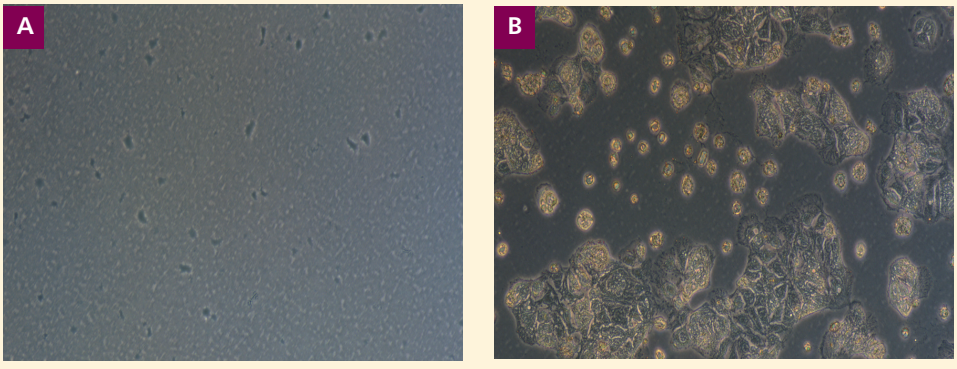


Figure 1: No adherent cells are present on vessels with cell-repellent surface. A: Cell culture dish with cell-repellent surface after incubation with EtOH. B: Cell culture dish with CELLSTAR® TC surface after incubation with EtOH.

greiner bio-one

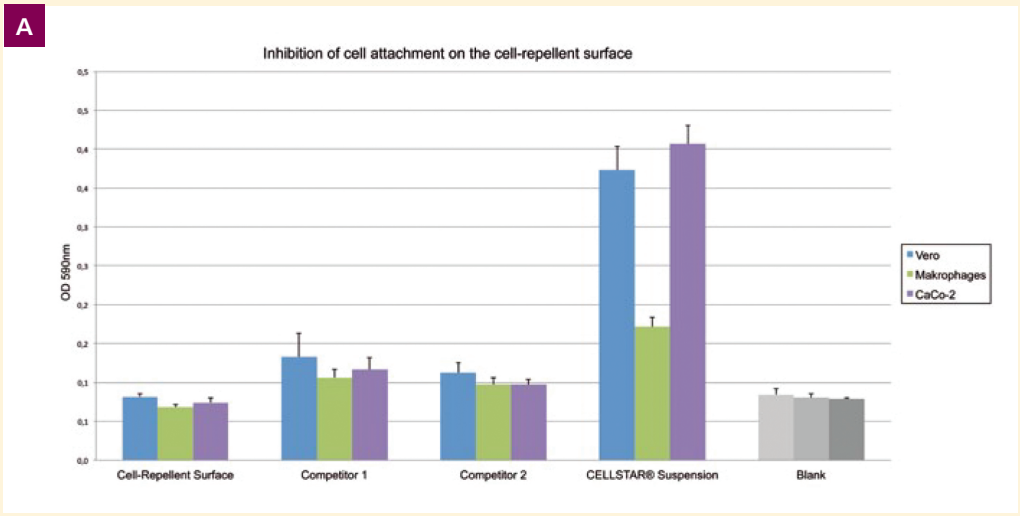


Figure 2: The cell-repellent surface inhibits cell attachment effectively. A) Spectroscopic analysis ofcell attachment of Vero, $CaC0_2$ cells and macrophages.Cells were seeded in Flat-bottom 96-well microplates, incubated at 37°C and 5% CO_2. After 24 hours the media were discarded. Cell attachment was analyzed by crystal violet staining. After dissolving the crystal violet dye in the cells attached to the well surface, optical density was measured at 590nm. B) Microscopic analysis with 10 x magnification after crystal violet staining (Left: CELLSTAR suspension; Right: cell-repellent surface).

generally used. With semi-adherent cell lines like macrophages or even adherent cell lines like Vero or $CaCO_2$ cells, this kind of surface does not reliably prevent cell attachment.

If cultivated in vessels with a cell-repellent surface, these cell lines exhibit an almost total inhibition of attachment (Fig. 2). Tested cell lines are listed in Table 1.

Culture of Spheroids and Stem Cell Aggregates

In pharmaceutical and basic research, two-dimensional (2D) cultures are still predominant. Nevertheless, 2D cultures can only mimic to a limited extent the conditions in physiological tissue where cells are able to interact in a three-dimensional network. Therefore, results generated from 2D cultures have often limited relevance for clinical effectiveness[1].

The employment of spheroid cultures plays an important role as an alternative approach to better mimic physiological conditions, especially in cancer research. Spheroids are self-assembled spherical cell clusters with different zones (Fig. 3). These zones are characterized by metabolic and proliferative gradients resembling the physical conditions in avascular tumors or micrometastases[2,3]. As a consequence, experimental data obtained with spheroids are often more significant than data from 2D cell culture experiments. Pluripotent stem cells with their ability to differentiate into cell types of all germ layers (endoderm, mesoderm and ectoderm)[4] hold great promise for drug development and therapeutic applications, as well as for basic research.

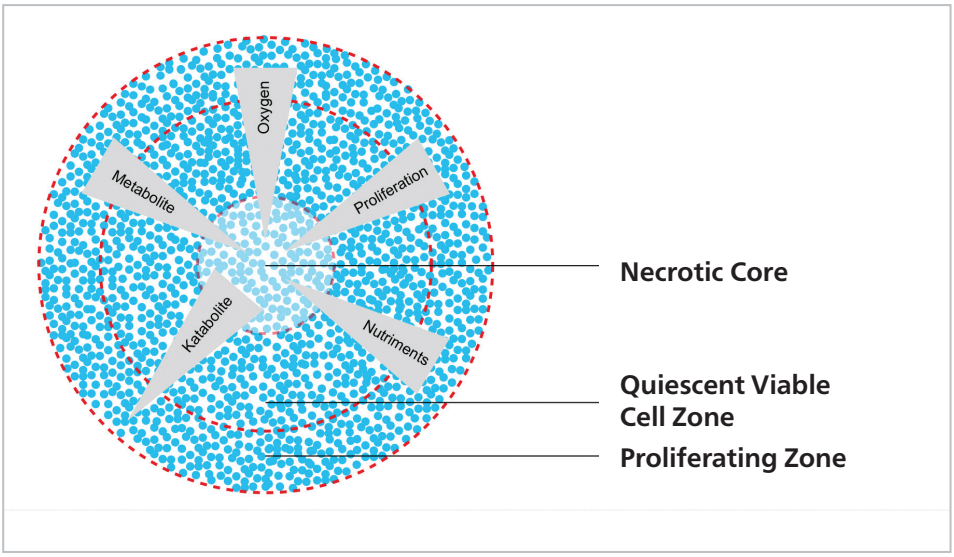
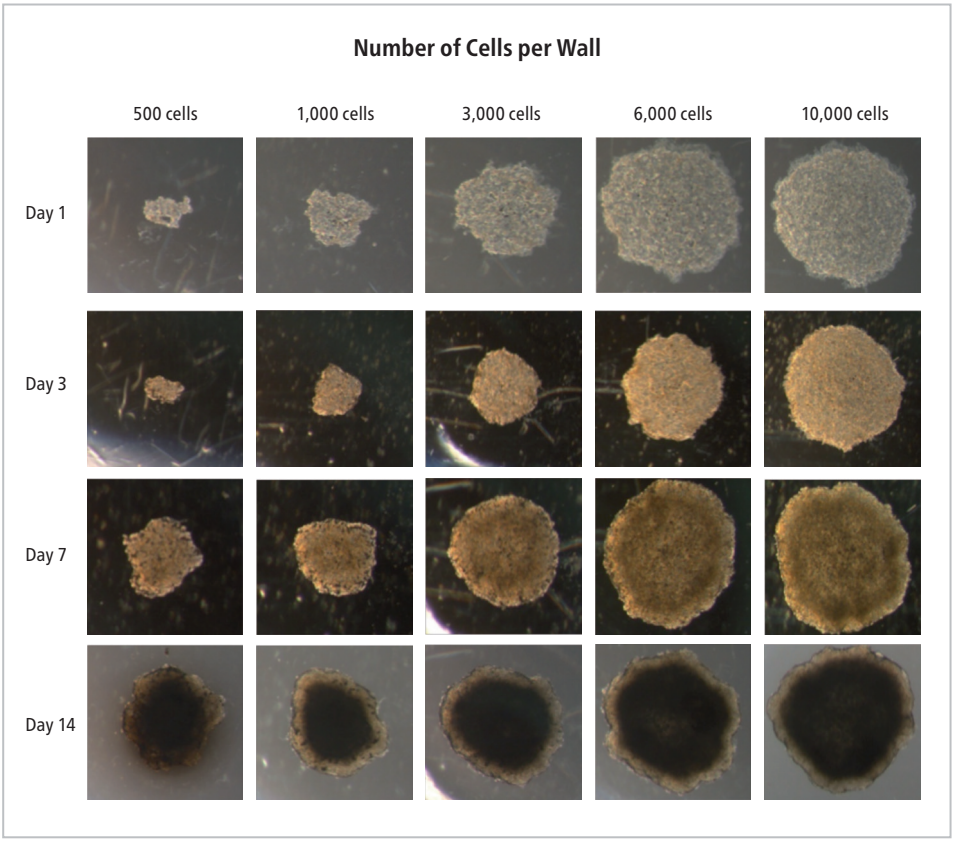


Figure 3: Schematic description of a tumor spheroid: nectrotic core, quiescent viable cell zone, and proliferating zone.



A key step for the cultivation of stem cells is the formation of non-adherent cell aggregates, called embryoid bodies[5]. Pluripotent cell types ,which are able to form embryoid bodies, comprise both embryonic stem cells and induced pluripotent stem cells.

For the formation of spheroids and embryoid bodies cell-cell interaction has to be dominant over the interaction of the cells with the surface of the culture vessel used. Standard cultivation approaches comprise the hanging drop method, spinner flask, or rotary cell culture for spheroid cultivation[1] and static suspension culture for embryoid bodies. However, these approaches have only limited compatibility with automation and high-throughput screening.

On the other hand, 'classic' cell culture vessels like microplates fulfill all the necessary demands for automated handling and imaging. If equipped with a surface effectively preventing cell-surface interactions like the Greiner Bio-One cell-repellent surface, these vessels represent a perfect platform for cultivating spheroids (Fig. 4) and stem cell aggregates (Fig. 5, 6). Tested cell lines for spheroid and aggregate formation are listed in Table 1.

References

For a complete list of references, visit ***vwr.com/vwrbiomarke.***

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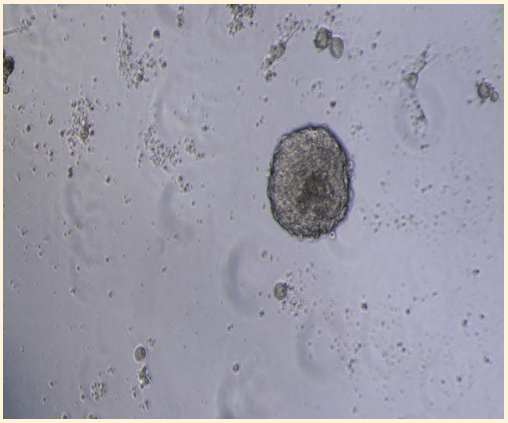


Figure 5: Aggregates of murine ES-D3 cells grown for 10 days in a 96-well Flat-bottom microplate with cell-repellent surface. Before the transfer to the cell-repellent surface, cells were cultivated on feeder cells and detached with accutase.

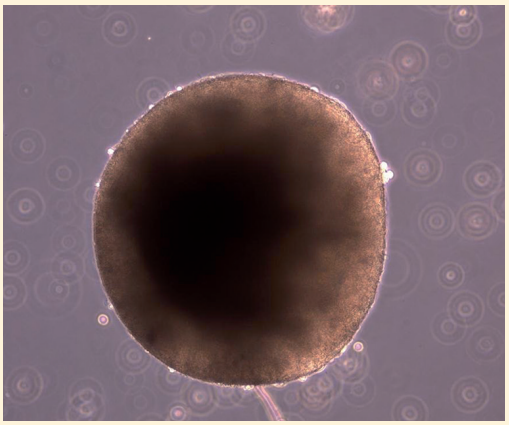


Figure 6: Aggregate formation of human induced pluripotent stem cells (iPSCs) cultured in a 96-well U-bottom microplate with cell-repellent surface. Before the transfer to the cell-repellent surface, cells were cultivated on Matrigel™ and detached with accutase.

Description	Cat. No.	Unit
Cell Culture Flask, White Filter Screw Cap, 250 mL	10836-541	Case of 15
Cell Culture Flask, Flat Flask Design, White Filter Screw Cap, 550 mL	75800-006	Case of 5
6-Well Microplate with Lid, PS, Flat-Bottom, Clear, Sterile	30618-022	Case of 5
24-Well Microplate with Lid, PS, Flat-bottom / Chimney Well, Clear, Sterile	10014-320	Case of 5
96-Well Microplate with Lid, PS, Flat-Bottom, Black µClear, Sterile	10014-318	Case of 32
96-Well Microplate with Lid, PS, Round (U)-Bottom, Clear, Sterile	76199-200	Case of 32
384-Well Microplate with Lid, PS, Flat-Bottom, Black µClear, Sterile	76199-198	Case of 32
384-Well Microplate with Lid, PS, Round (U)-Bottom, Clear, Sterile	10014-326	Case of 32

Organoids

Mini Organs in a Dish

Organoid models include three-dimensional (3D) cell culture systems that closely resemble the *in vivo* organ or tissue from which they are derived. These 3D systems replicate the complex spatial morphology of a differentiated tissue and allow biologically relevant cell-cell and cell-matrix interactions; ideally, sharing similar physiological responses found within *in vivo* differentiated tissues. This is unlike traditional two-dimensional (2D) cell culture models that often bear little physical, molecular, or physiological similarity to their tissue of origin.

Although the earliest 3D organoid models were first described over 40 years ago, their utility has remained limited until recently. Early organoid models required large numbers of starting cells, were not amenable to high-throughput screening, and often exhibited limited *in vivo* viability[1]. These drawbacks have now been largely eliminated, as advances in multipotent stem and progenitor cell isolation have allowed researchers to develop highly reproducible, long-lived organoids.

The rapid developments in organoid technology, and the wide usage of the term organoid for a variety of both *in vivo* and *in vivo* structures, led Lancaster and Knoblich to suggest a basic definition for organoids. They defined organoid as: "A collection of organ-specific cell types that develops from stem cells or organ progenitors and self-organizes through cell sorting and spatially restricted lineage commitment in a manner similar to *in vivo*". According to Lancaster and Knoblich, an organoid should possess several important features characteristic to the respective organs: "(1) it must contain more than one cell type of the organ it models; (2) it should exhibit some function specific to that organ; (3) the cells should be organized similarly to the organ itself"[2].

In 2009, Hans Clevers and Toshiro Sato used adult stem cells from mouse intestine to create the first mini-gut organoids from murine cells[3] and later extended their method to human epithelial organoids[4]. These organoids were expected to allow researchers to gain new insights into the biology of gut health and disease, including colorectal cancer.

This method inspired many other scientists to create a variety of organoids from mouse and human tissues. These clumps of cells are small enough to survive without blood supply, yet large and complex enough to teach us something about tissue and whole-organ development and physiology.

A typical organoid protocol starts with isolated embryonic or pluripotent stem cells, which are then cultured in a supporting scaffold (such as Corning® Matrigel®) that enables three-dimensional growth. Organoids are comprised of multiple differentiated cell types that are found in the relevant organ *in vivo*. For example, all cell types of the intestinal epithelium are represented in the Matrigel-based model described by Sato et al[3]. The signaling pathways governing organoid formation were found to be identical to those used during *in vivo* organ development and homeostasis; thus, cytokines, growth factors, and small molecules were also included in the culture medium in order to activate or inhibit specific signaling pathways. Even tissues that are closely related, such as the small intestine and colon, require different combinations of signaling molecules in the process of organoid formation[4].

There are different ways to obtain an organoid culture, and some of these are just beginning to be explored. Below you will find some examples of various organoid models that have been developed, with an emphasis on the

cytokines, growth factors, and small molecules that were used.

Gastrointestinal (GI) Organoids

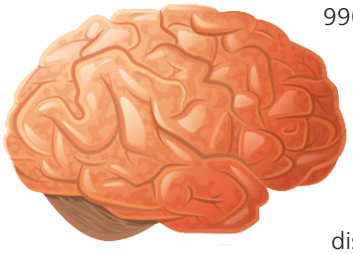
Historically, preclinical GI medical research has relied entirely on animal models and cancer cell cultures that are of limited relevance to human physiology; thus, the ability to obtain GI organoids from human cells is of great importance[4, 5].

The intestinal epithelial layer is made up of tiny, slender projections, called villi. The niches formed at the bases of the villi, known as crypts, are home to the intestinal stem cells responsible for constant renewal of the intestinal lining. In the original study that generated murine small intestine organoids, epidermal growth factor (EGF), R-Spondin-1, and Noggin were included in the medium[3]; whereas a later study that demonstrated the formation of murine colon organoids added Wnt-3a to the above three growth factors. The generation of small intestine and colon organoids from human cells also required two small molecules, TGF-β inhibitor (A 83-01) and p38 MAP kinase inhibitor (SB 202190), in addition to the above mentioned cytokines[4].

In a recent study, human intestinal organoids (HIO) that were produced *in vitro* from human embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) were engrafted *in vivo* and became functioning mature intestinal tissue. For induction of definitive endoderm (DE), human ES or iPS cells were treated with medium containing Activin A, followed by a medium containing Activin A, FGF-4, and the GSK3 inhibitor (CHIR 99021) to form spheroids. The spheroids were then plated in Matrigel, and maintained in intestinal growth medium supplemented with EGF and Noggin to generate the HIO that were later engrafted into immunodeficient mice[6].

Brain Organoids

The complexity of the human brain, which made it difficult to study many brain disorders in model organisms, called for the establishment of an *in vivo* model of human brain development.



A protocol for generating 3D brain tissue, or so-called cerebral organoids, that closely mimics the endogenous developmental program used patient-specific iPS cells to form a 3D organoid culture model of microcephaly, which is a disorder that had been difficult to reproduce in mice. iPSCs, resulting from reprogrammed patient skin fibroblasts, were incubated in a medium supplemented with FGF-basic, CHIR 99021, and MEK inhibitor (PD 0325901) for 21 days. The outgrowing colonies were picked and passaged on inactivated CF-1 mouse embryonic fibroblasts (MEFs). Later, single cells were plated in media containing low levels of FGF-basic and high levels of ROCK inhibitor (Y 27632). The neuroepithelial tissues that were formed were transferred to droplets of Matrigel, and after a period of stationary growth these tissue droplets were transferred to a spinning bioreactor containing differentiation medium supplemented with retinoic acid. Analysis of these patient organoids demonstrated premature neuronal differentiation, which could explain the disease phenotype[7]. Recently, a miniaturized spinning bioreactor (SpinΩ) was developed to generate forebrain-specific organoids from human iPSCs. Detached human iPSC colonies were transferred on day 1 to a 6-well plate with stem cell medium containing A 83-01. On days 5-6, half of the medium was replaced with induction medium containing Wnt-3a, CHIR 99021, and a selective TGF-β inhibitor (SB 431542). On day 7, organoids were embedded in Matrigel and grown in induction medium for 6 more days. On day 14, organoids were dissociated from Matrigel and 10–20 organoids were transferred to wells of a 12-well spinning bioreactor (SpinΩ) containing differentiation medium. At day 71, differentiation medium was changed to maturation medium, containing BDNF, GDNF, and TGF-β1. The organoids could grow beyond 110 days in maturation medium with medium change occurring every other day. These organoids were used to study the effects of Zika virus exposure on the brain and could be employed in the future for drug testing. In addition, this platform was also used to generate midbrain and hypothalamus organoids from human iPSCs[8].

B Cell Follicle Organoids

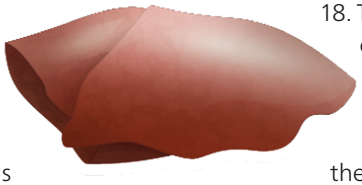
When naive B cells encounter antigens, they form clusters of cells called germinal centers in a lymph node or in the spleen; here they proliferate, mutate to produce high-affinity antibodies, and undergo clonal expansion. Until now, recreating this process using 2D cultures *in vitro* was difficult.

Instead of using the conventional Matrigel for 3-D culture, a gelatin and silicate-nanoparticle mix that mimics the environment of the body's lymphoid organs was developed. Naïve B cells

obtained from splenocytes were co-cultured with engineered stromal cells expressing both CD40L and B cell activating factor (BAFF) in a medium containing murine IL-4. Much faster than in 2D cultures, the B cells in these organoids matured and displayed class switching within days[9].

Liver Organoids

Liver development involves an intricate interaction of tissues derived from both the endoderm and mesoderm. The liver is initially derived from endoderm hepatic bud structures, which develop from foregut epithelium. The hepatoblasts derived from hepatic buds contribute hepatocytes and biliary epithelium, whereas liver fibroblasts and stellate cells originate from nearby mesoderm-derived mesenchyme[2, 10]. A recent approach that was established to generate human liver bud-like tissues employed a mixture of three cell populations, mimicking the early cell lineages of the developing liver: human PSC-derived hepatic cells, human mesenchymal stem cells, and human endothelial cells. For endodermal differentiation, human iPSCs were seeded on a Matrigel-coated dish in medium containing Activin A. Human iPSC-derived endodermal cells were then treated with a medium containing human FGF-basic and BMP-4 for differentiation of hepatic endoderm cells (iPSC-HEs). Human iPSC-HEs were then cultivated with two stromal cell populations: human umbilical vein endothelial cells (HUVECs) and human mesenchymal stem cells (MSCs). The cells spontaneously formed 3D liver buds when mixed at a high density on a layer of Matrigel. When these liver buds were transplanted into mice they displayed vascularization and showed liver-specific functions, and transplanted mice survived the drug-induced lethal liver failure model[2, 11].



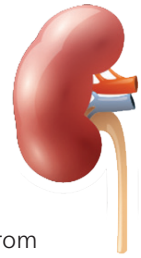
Retina Organoids

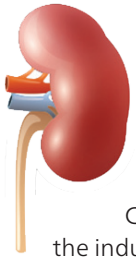
The eye is a highly complexed organ and consists of a variety of cells that are combined in an organized three-dimensional fashion. The light-receptive neural region of the eye, the retina, is derived from the neural ectoderm. Two adjacent epithelial layers are formed early in retinal development: the outer retinal pigmented epithelium and the inner neural retina, which eventually become a tissue containing layers of photoreceptors and supportive cell types[2, 12].

Optic cup organoids were generated from human ESCs, and were compared to similar organoids generated from mouse ESCs. For retinal differentiation, hESCs were reaggregated in retinal differentiation medium containing Y 27632. Matrigel was added from day 2 to day 18. For optic cup formation, CHIR 99021 (or recombinant Wnt-3a) and recombinant human Sonic Hedgehog (Shh) were added to the differentiation medium from day 15 to day 18. These human retinal organoids shared many characteristics displayed by mouse retinal organoids; however, they showed several human-specific differences. In particular, the human retinal organoids were larger than mouse organoids, they developed more slowly, and they grew into tissue comprising multilayers that contained both rods and cones (cone differentiation is rare in mouse ESC culture)[2, 13].

Kidney Organoids

The kidney differentiates from the intermediate mesoderm (IM) through the interaction of IM-derived metanephric mesenchyme (MM) and a formed ureteric bud (UB). Nephron progenitors derived from the MM are the source of nephrons, while the IM itself is derived from the posterior primitive streak[14, 15].





Human ESCs that were cultured on irradiated mouse embryonic fibroblast feeder cells were plated in a Matrigel-coated 96-well plate. After overnight culture, cells were exposed to BMP-4 and Activin A, or alternatively to CHIR 99021, in a serum-free media, then cultured in FGF-9 and heparin- containing media to induce IM cells. These cells were subsequently incubated in a medium containing FGF-9, BMP-7 and retinoic acid (in the case of BMP-4/Activin A-induced cells), or FGF-9 and heparin (in the case of CHIR 99021 induced cells). For the induction of kidney organoids, hESC-derived kidney cells were dissociated into single cells, spun down to form a pellet, placed onto a filter membrane with a collagen IV coat, and, lastly, floated on culture media. This study successfully differentiated hESCs under chemically-defined culture conditions, using growth factors that participate in normal embryogenesis, and resulted in coordinated generation of UB and MM that finally formed, *in vivo*, self-organizing 3D structures, including nephron formation[15].

The Therapeutic Potential of Organoids

A wide array of organoids from organs of all three embryonic layers have been studied, and include: endoderm-derived organoids of thyroid, lung, pancreas, liver, stomach, and intestine; mesoderm-derived organoids of heart, skeletal

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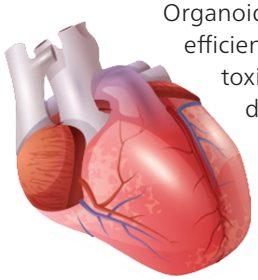
muscle, bone, and kidney; and ectoderm-derived organoids of retina, brain, pituitary, mammary gland, inner ear, and skin[2].



The major focus of future organoid studies will continue to investigate and refine the developmental processes and most likely, will subsequently advance into disease modeling. Organoids may be useful to further research in developmental disorders, genetic conditions, cancer, and degenerative disease, to name a few[2, 16].

Utilizing patient iPSCs will allow valuable disease modeling, especially when adequate animal models are not available. Organoids can also be used for more efficient testing of drug efficacy and toxicity by removing discrepancies due to the differences between animal and human cells. Organoid drug testing might also dramatically reduce the use of animals for pre-clinical trials. The hope is that organoids are another step in the long journey towards *in vivo* construction of tissues and organs for transplantation into patients; however, many obstacles still need to be addressed along the way, such as proper maturation and the lack of vascularization.

References

For a complete list of references, visit ***vwr.com/vwrbiomarke.***

Description	Size	Cat. No.	Unit
Human/Murine/Rat Activin A	10 µg	**10772-506**	**Each**
Human/Muire/Rat BDNF	10 µg	**10781-154**	**Each**
Human BMP-4	5 µg	**10779-136**	**Each**
Animal-Free Human EGF	100 µg	**10781-692**	**Each**
Human GDNF	10 µg	**10781-220**	**Each**
Human Sonic Hedgehog	25 µg	**10779-016**	**Each**
CHIR 99021	10 mg	**75844-610**	**Each**
ROCK inhibitor (Y 27632)	10 mg	**75844-614**	**Each**

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FB Essence	500 mL	10803-034	Each
FB Essence, Heat Inactivated	50 mL	10799-384	Each
FB Essence, Heat Inactivated	500 mL	10799-390	Each

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100 L/min	Contact	76076-708	Each
100 L/min	Petri	76169-646	Each
200 L/min	Contact	76076-710	Each
200 L/min	Petri	76169-648	Each

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Size	Cat. No.	Unit
0.3 mL	**10766-890**	**Each**
0.75 mL	**10766-892**	**Each**
1.5 mL	**10766-888**	**Each**
5 x 1.5 mL (5-pack)	**10767-126**	**Each**
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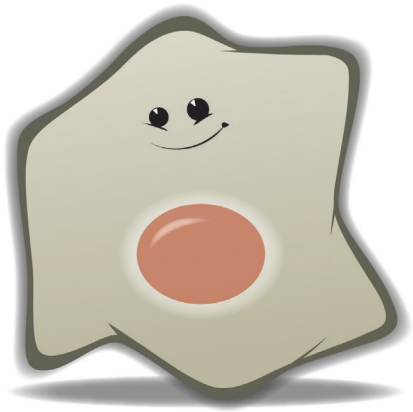

Description	Size	Cat. No.	Unit
M2-Macrophage Generation Medium, DXF (Ready-to-Use)	250 mL	**10172-406**	**Each**
M1-Macrophage Generation Medium, DXF (Ready-to-Use)	250 mL	**10172-408**	**Each**
Macrophage Base Medium, DXF	250 mL	**10172-410**	**Each**
Monocyte Attachment Medium (Ready-to-Use)	250 mL	**10172-398**	**Each**
Macrophage Detachment Solution, DXF	250 mL	**10175-308**	**Each**
PromoFectin-Macrophage	0.1 mL	**10181-152**	**Each**
PromoFectin-Macrophage	0.5 mL	**10181-154**	**Each**

CytoFLEX System Performance Evaluation

By James Tung, Jesus Lemus, Zhao Jing, Domenic Fenoglio, and Paul Scibelli (Beckman Coulter, Inc, Miami, FL, USA); Kathy Regheb, Jennifer Sturgis, and Paul Robinson (Purdue University Cytometry Laboratories); Dan Condello, Erika Duggan, and John Nolan (La Jolla Bioengineering Institute); Albert Donnenberg (University of Pittsburgh Cancer Center); and Yijun Huang (Sun Yat-Sen University, School of Medicine, Guangzhou, China)

ABSTRACT

Performance testing of cytometry instruments is utilized to characterize the capability of the cytometer to perform high complexity applications. We have tested the new CytoFLEX flow cytometer against the performance measurements for sensitivity, dynamic range, population resolution, linearity, stability, reproducibility, and small particle resolution. Commercially available bead standards were used to evaluate performance expectations across multiple parameters.

The CytoFLEX flow cytometer was evaluated at La Jolla Bioengineering Institute, Purdue University Cytometry Laboratory (PUCL) and the Beckman Coulter, Miami site. Additional data was collected through a collaborative effort with Al Donnenberg from University of Pittsburgh Cancer Center and School of Medicine at Sun Yat-Sen University, Guangzhou, China. Each instrument was equipped with three lasers (405nm, 488nm and 638nm) and 12 parameters with 9 fluorescence detectors. Instruments were equipped with the enhanced Violet laser Side Scatter Channel (VSSC) option for small particle detection.

MATERIAL AND METHODS

Materials

CytoFLEX multicolor flow cytometer equipped with enhanced Violet laser Side Scatter Channel (VSSC) option (manufactured by Beckman Coulter).

Spherotech SHPHERO™ 8 Peak Rainbow Beads were used for resolution, linearity, stability, and separation testing.

To calculate the daily Quality Control rCV (robust CV) through the CytExpert Software, CytoFLEX QC Beads were used. The QC beads were collected on three different CytoFLEX instruments. Robust CV is approximately the 75th percentile minus the 25th percentile divided by the median and is not as skewed by outlying values as the CV.

Results

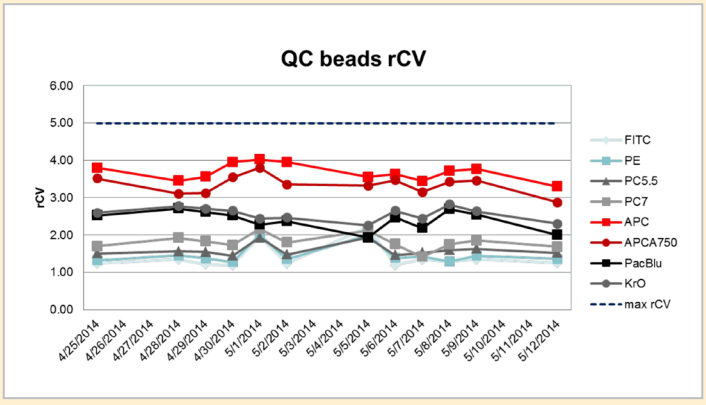

Figure 1 (above). rCV analysis of QC beads over 18 days. QC beads were run on the instrument for 18 days. Twelve startup and shutdown cycles were captured and rCV were tracked via Levey-Jennings. The data below is relative parameters for each laser.

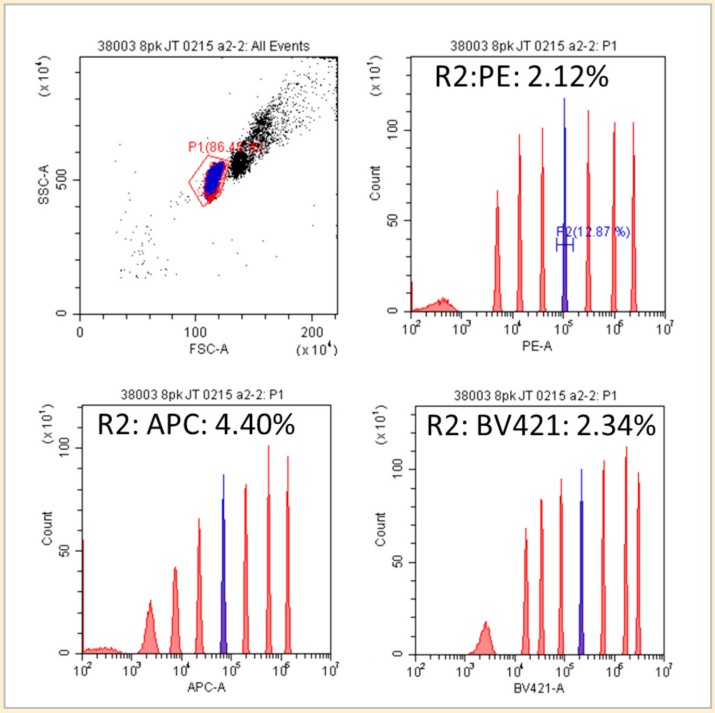

Figure 2 (right). rCVs on 8 peak beads run continuously for one hour period. Spherotech 8 peak rainbow beads were run continuously for one hour on the CytoFLEX unit. rCV data was collected on Peak No. 5 PE: 2.12%, APC: 4.40% and BV421: 2.34% showing the stability of the system.

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For Microparticle testing the following were used:

Laboratory 1: Beckman Coulter Miami Spherotech beads. 0.5µm and 0.2µm.

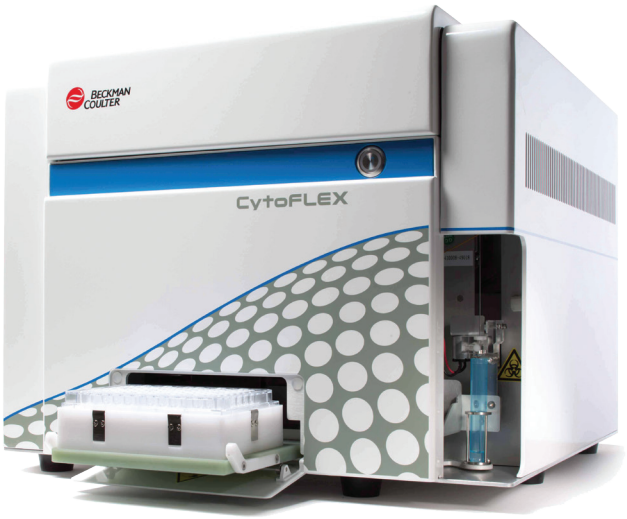
Laboratory 2: La Jolla Bioengineering Institute
Polybead Polystyrene Sampler Kit III PolySciences (16905-1)
Polybead Polystyrene Sampler Kit II PolySciences (21756-1)

Laboratory 3: PUCL.
PolySciences beads 0.5, 0.4, 0.35, 0.3, and 0.2µm beads

Laboratory 4: School of Medicine, Sun Yat-Sen University, Guangzhou, China
Beckman Coulter Particle Sizing Standards 0.5µm, 0.3µm, and 0.2µm latex beads (non-fluorescent) mixture for testing. Beads were diluted using UPW (UltraPure Water). Cytometer comparison was conducted against a competitor system configured with equivalent lasers and filter setup.

Separation index is described as the separation of adjacent peaks of Rainbow beads measured by the difference between the peak MFIs, divided by the geometric mean of their standard deviations. Separation Index is measured using the following formula:

$$\text{Separation Index} = \frac{(\text{MFIpeak2}-\text{MFIpeak1})}{(\text{SQRT}(\text{SDpeak1}^2+\text{SDpeak2}^2)/2)}$$



RESULTS

(See below and on page 18)

SUMMARY

Performance evaluation results show the CytoFLEX cytometer is stable, reproducible and sensitive especially in violet and far red channels. The system's ability to separate populations as measured by the separation index is overall superior to comparable instrumentation. Small particle evaluation showed clear resolution of 0.2µm particles from noise using SSC from the Violet laser. Moreover, the CytoFLEX flow cytometer can clearly distinguish between different sizes of submicron beads.

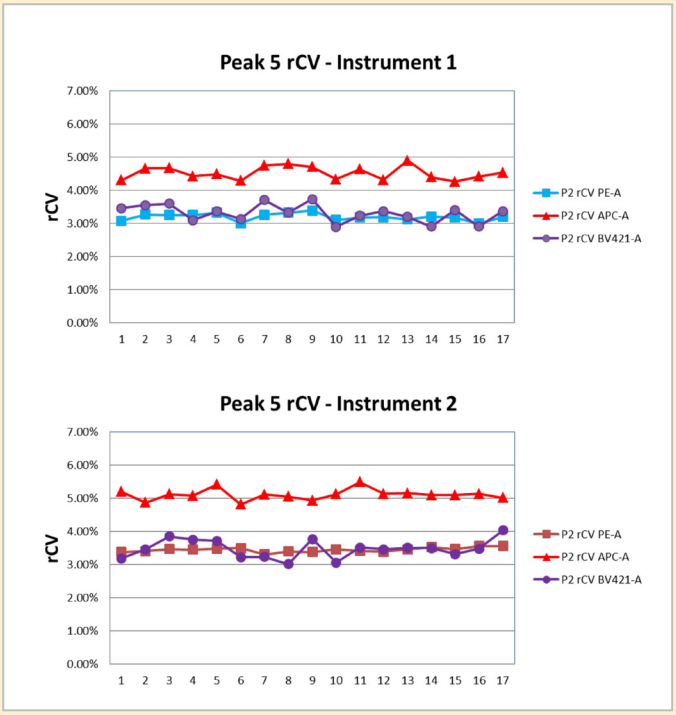


Figure 3. Spherotech 8 peak rainbow beads were run every day for 17 days on two instruments and the rCV Data was collected and plotted on Peak No. 5.

Description	Cat. No.	Unit
CytoFLEX System, B4-RO-VO	76183-344	Each
CytoFLEX System, B3-R1-VO	76183-346	Each
CytoFLEX System, B2-RO-V2	76183-348	Each
CytoFLEX System, B2-R2-VO	76183-350	Each

Results

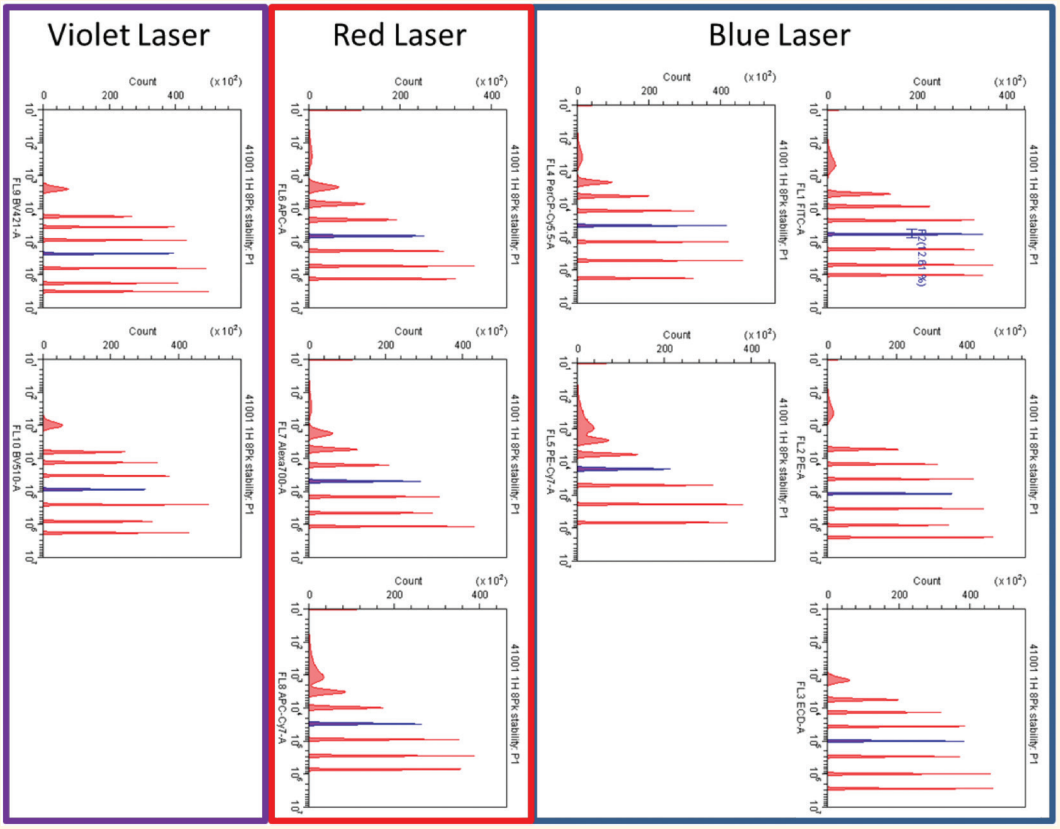


Figure 4. Spherotech 8 peak rainbow bead data for 3 laser 10 color detectors. *Data provided by University of Pittsburgh Cancer Center*

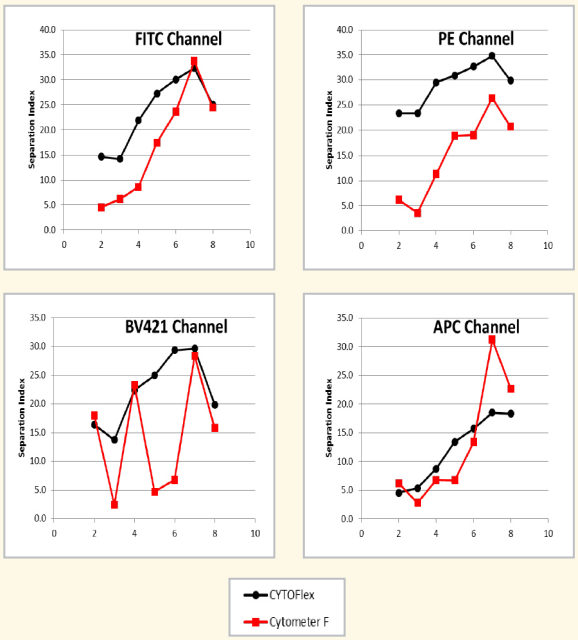


Figure 5. Spherotech 8 peak rainbow bead data calculated for a Separation Index between bead peaks as compared to a standard high complexity research cytometer. *Data provided by University of Pittsburgh Cancer Center*

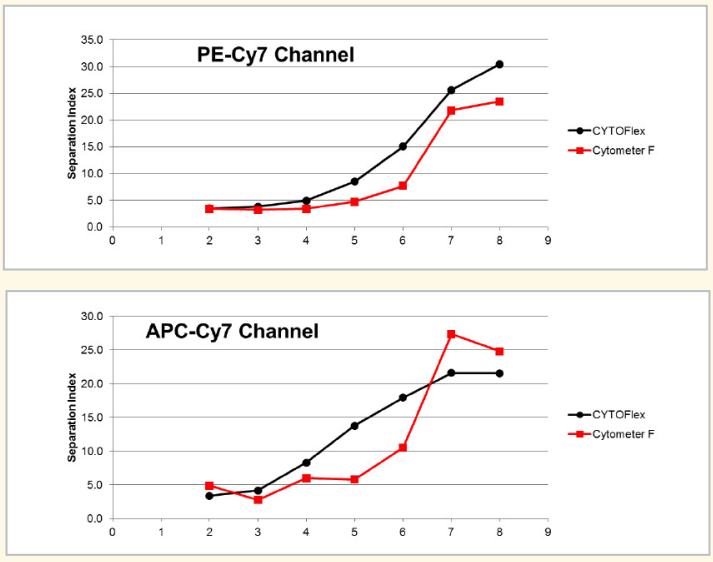


Figure 6. Spherotech 8 peak rainbow bead data calculated for a Separation Index between bead peaks as compared to a standard high complexity research cytometer. Data is Specific for Red Channel Sensitivity. *Data provided by University of Pittsburgh Cancer Center*

Results

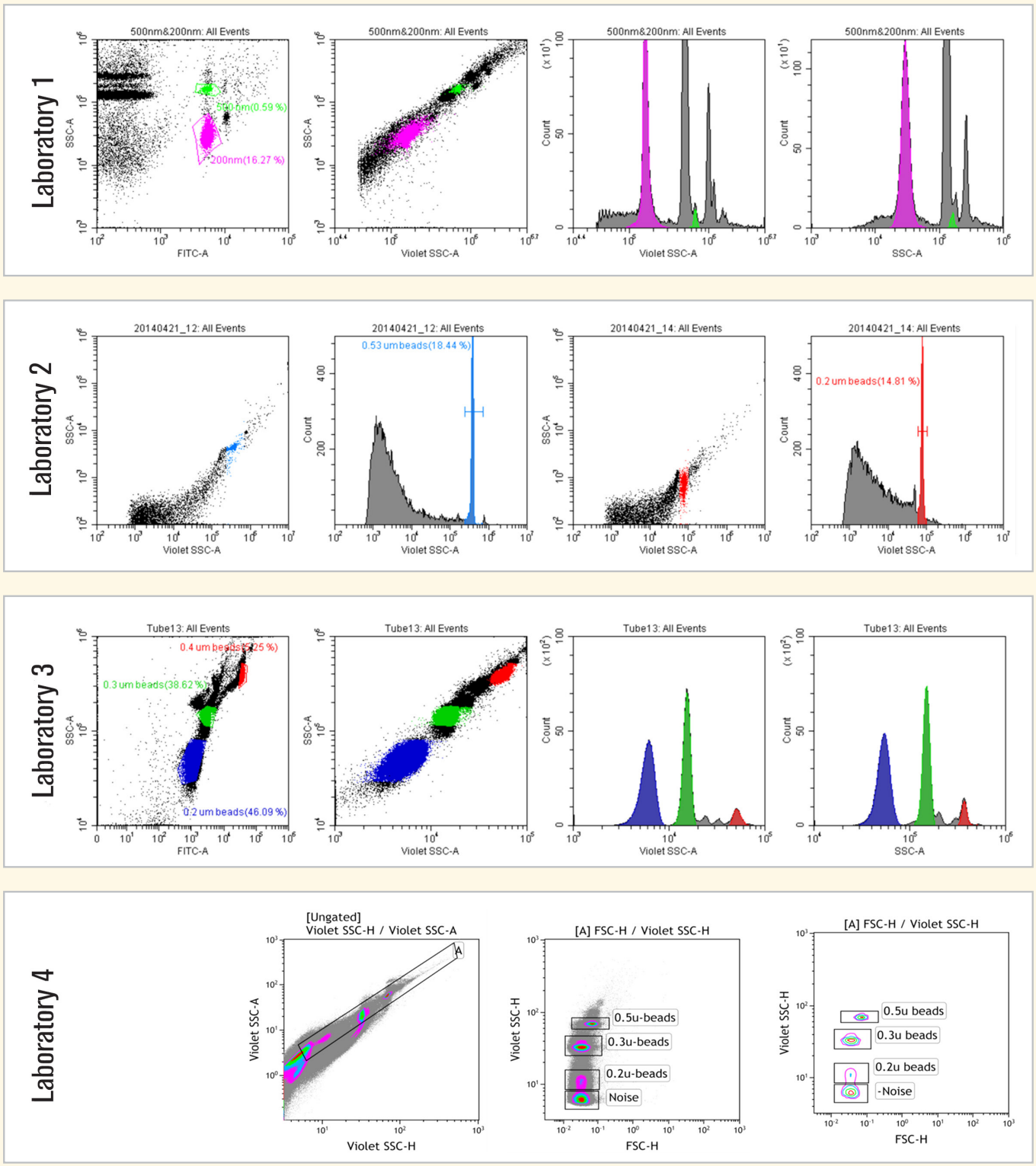


Figure 7. Microparticle Detection. Four separate labs ran bead based small particles detected using side scatter and VSSC. *Data provided by University of Pittsburgh Cancer Center, PUCL, La Jolla Bioengineering Institute, Sun Yat-Sen University Medical College.*

NucView® Caspase-3 Substrates

The Only Caspase Substrates for Real-Time Apoptosis Detection

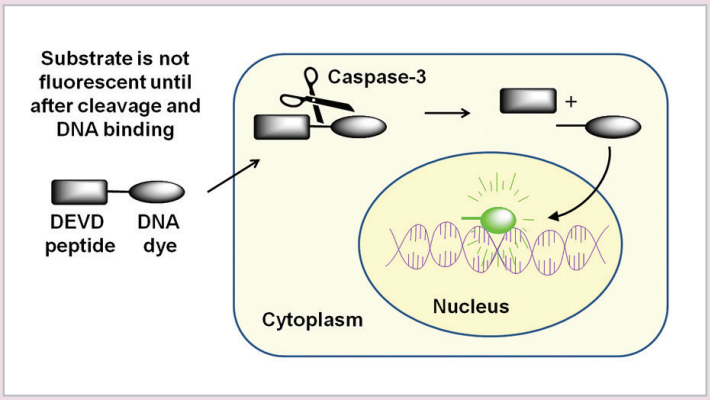


Figure 1. Schematic illustrating the mechanism of NucView Caspase-3 Substrates.

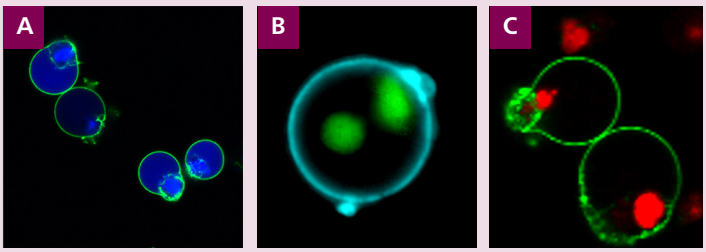


Figure 2. Apoptotic HeLa cells stained with (A) NucView 405 caspase-3 substrate (blue) and CF488A Annexin V (green), or (B) stained with NucView 488 caspase-3 substrate (green) and CF647 Annexin V (blue). (C) Apoptotic MCF-7 cells stained with NucView 530 Caspase-3 Substrate (red) and CF488A Annexin V (green). NucView substrates stain nuclei of apoptotic cells and CFdye Annexin V shows plasma membrane staining. Cells were imaged using an Olympus epifluorescence microscope with CCD camera.

Proteolysis of cellular substrates by caspase-3 results in the morphological and biochemical features of apoptosis[1]. NucView caspase-3 substrates are novel cell membrane-permeable fluorogenic caspase substrates designed for detecting caspase-3 activity in real time[2].

Traditional fluorogenic caspase substrates[3] require cell lysis and cannot be used to measure caspase activity in live cells; furthermore such assays measure only the average caspase activity in a cell population. Fluorescently labeled caspase inhibitor assay (FLICA) reagents can enter live cells to detect caspase activity[4], but because the fluorescent probes are irreversible caspase inhibitors, they cannot be used to follow caspase activity in real time.

NucView caspase-3 substrates are novel fluorescent probes for the detection of caspase-3 activity within intact cells. The substrate consists of a fluorogenic DNA dye and a DEVD caspase-3/7 substrate moiety. The substrate, which is initially non-fluorescent and nonfunctional as a DNA dye, crosses the cell membrane to enter the cytoplasm, where it is cleaved by caspase-3 to form a high-affinity DNA dye. The released DNA dye migrates to the cell nucleus to stain the nucleus with bright fluorescence (Figure 1). NucView caspase-3 substrate staining can be detected by fluorescence microscopy, flow cytometry, microplate imaging systems, or live cell imaging systems like the IncuCyte® Live Cell Analysis System. For microscopy applications, the substrate is bi-functional, allowing the visualization of nuclear morphology in apoptotic cells in addition to detection of caspase-3 activity (Figure 2).

NucView substrates do not interfere with caspase-3 activity[2], allowing real time monitoring of fluorescence kinetics over time frames of hours to days[5,6]. Staining can be detected in as little as 15 minutes in cell culture medium with no washing required, making it compatible with high-throughput and high content screening. After staining with NucView substrates, cells can be fixed in formaldehyde and processed for subsequent analysis with immunostaining[7]. Detection of caspase-3 activity using NucView substrates has been reported for a wide variety of immortalized and primary animal and plant cells (Table 1 and Table 2).

NucView 488 and MitoView 633 Apoptosis Kit

The loss of mitochondrial membrane potential is an early event in apoptosis, preceding phosphatidylserine externalization and coinciding with caspase activation[8]. The NucView 488 and MitoView 633 Apoptosis Kit includes include far-red fluorescent MitoView 633 mitochondrial membrane potential dye for simultaneous measurement of caspase-3 activity and mitochondrial membrane potential by fluorescence microscopy or flow cytometry (Figure 3).

Dual Apoptosis Assay with NucView 488 Caspase-3 Substrate and CF® Dye Annexin V

Annexin V is a 35–36kDa protein that has a high affinity for phosphatidylserine (PS). During apoptosis, PS is translocated from the inner to the outer leaflet of the plasma membrane, triggering engulfment of the dying cell by phagocytes[9]. The Dual Apoptosis Assay kit with NucView 488 caspase-3 substrate is available with a choice of red (CF594) or far-red (CF640R) CF® dye

Biotium

Cell line	Species	Cell type	References
293-H	Human	Embryonic kidney	10
293-T	Human	Embryonic kidney	11
67NR	Mouse	Mammary carcinoma	12
A172	Human	Glioma	13
A204	Human	Sarcoma	14
BeWo	Human	Trophoblast	15
CCL-134	Human	IPF pulmonary fibroblast	16
CCL-190	Human	Pulmonary fibroblast	16
GE11	Mouse	Epithelial	17
HeLa	Human	Cervical cancer	15, 18, 2
HMEC	Human	Microvascular endothelial	5
HT-1080	Human	Breast fibrosarcoma	19, 14
Jurkat	Human	T-lymphocyte	2, 20
K562	Human	Myelogenous leukemia	21
MCF-7	Human	Breast adenocarcinoma	22
MDS-MB-231	Human	Breast adenocarcinoma	22
MES-SA	Human	Uterine sarcoma	14
MES-SA/DX	Human	Uterine sarcoma	14
Min 6	Mouse	Pancreatic insulinoma	23
N19	Mouse	Oligodendrocyte	24
NRK	Rat	Kidney epithelial	25
NRK-52E	Rat	Kidney epithelial	26
PC12	Rat	Pheochromocytoma	25
RD	Human	Rhabdomyosarcoma	14
RINm5F	Rat	Insulinoma	27
SKLMS1	Human	Leiomyosarcoma	14, 19
SW684	Human	Fibrosarcoma	14
SW872	Human	Liposarcoma	14
TK6	Human	TK6	25
U2OS	Human	Osteosarcoma	7
U251	Human	Glioblastoma	28
U373 MG	Human	Glioblastoma-astrocytoma	29

Cell type	Species	References
Dendritic cells	Mouse	30
Embryonic fibroblast (MEF)	Mouse	31
Hippocampal neurons	Rat	32
Idiopathic pulmonary fibrosis fibroblasts	Human	16
Immature B cells	Mouse	33
Kidney epithelial cells	Mouse	34, 35
Macrophages	Mouse	30
Mammary epithelial cells (3-D cultures)	Mouse	36
SVZ neural progenitor cells	Rat	37
Oligodendrocytes	Mouse	38
Pancreatic acinar cells	Mouse	39
Pancreatic beta cells	Rat	27
Pancreatic islet cells	Mouse	40
Peritoneal macrophages	Mouse	41
Pollen tubes	Field poppy	42
Pulmonary artery endothelial cells	Human	43
Retinal pigmented epithelial cells	Human, mouse	44
Skin fibroblasts	Sand cat	45
Thymocytes	Mouse	46
Umbilical vein endothelial cells	Human	47

Table 1. (left) Reports of immortalized cell lines tested with NucView 488
Table 2. (above) Reports of primary cells tested with NucView 488

Annexin V conjugates for dual color detection of caspase-3 activity and Annexin V binding by fluorescence microscopy (Figure 2) or flow cytometry.

Biotium offers a variety of apoptosis research tools and accessory reagents, including a broad selection of Annexin V conjugates featuring our exceptionally bright and photostable CF Dyes, CF dye dUTP conjugates and kits for fluorescent TUNEL staining, and far-red nuclear stains RedDot™ 1 and RedDot 2 for live and dead cells, respectively. NucView caspase-3 substrates

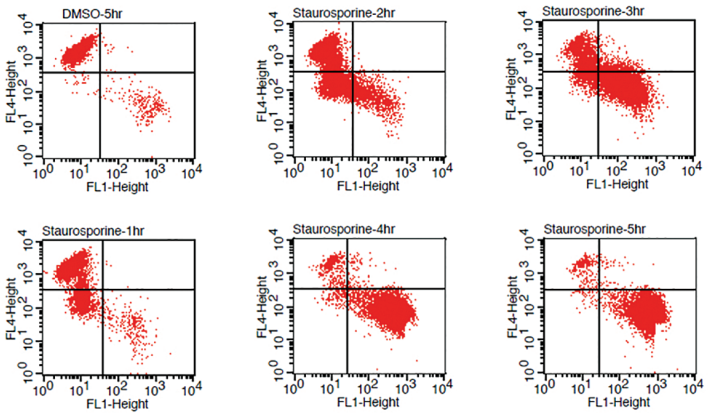


Figure 3. Flow cytometry analysis of caspase-3 activity and mitochondrial membrane potential using NucView 488 and MitoView 633 Apoptosis Kit. Jurkat cells were treated with 1µM staurosporine for the indicated times, then stained with the kit. Fluorescence was analyzed on a BD FACSCalibur™ flow cytometer. NucView 488 staining is plotted on FL1 (x-axis) and MitoView 633 staining is plotted on FL4 (y-axis). As apoptosis progresses, NucView 488 signal increases while mitochondrial membrane potential measured by MitoView 633 staining decreases.

are offered with three color choices, blue fluorescent NucView 405, green fluorescent NucView 488, and red fluorescent NucView 530. The substrates are provided as 1mM stock solutions in DMSO or PBS. DMSO facilitates NucView Caspase-3 substrate staining in some cell types (unpublished observations). The PBS stock is offered for use in DMSO-sensitive cell types. Biotium also offers the following apoptosis assay kits featuring NucView 488 substrate in combination with other apoptosis probes.

NucView Caspase-3 Substrate technology is covered by multiple US and international patents.

References

For a complete list of references, visit ***vwr.com/vwrbiomarke.***

Description	Size	Cat. No.	Unit
Dual Apoptosis Assay Kit with NucView 488 and CF640R-Annexin V	50 Assays	**71003-798**	**Each**
Dual Apoptosis Assay Kit with NucView 488 and CF594-Annexin V	50 Assays	**10098-604**	**Each**
NucView 488 and MitoView 633 Apoptosis Kit	100 Assays	**89411-556**	**Each**
NucView 488 and RedDot2 Apoptosis & Necrosis Kit	100 Assays	**89493-576**	**Each**
NucView Caspase-3 Substrates			
NucView 488 Caspase-3 Substrate, 1 mM in DMSO	100 µL	**71003-852**	**Each**
NucView 488 Caspase-3 Substrate, 1 mM in PBS	100 µL	**71003-844**	**Each**
NucView 405 Caspase-3 Substrate, 1 mM in DMSO	100 µL	**10154-178**	**Each**
NucView 530 Caspase-3 Substrate, 1 mM in DMSO	100 µL	**10001-596**	**Each**

Tuning the Elastic Moduli of Corning® Matrigel® and Collagen I 3D Matrices by Varying the Protein Concentration

CORNING

By Katie Slater, Jeff Partridge, and Himabindu Nandivada, Corning Incorporated, Life Sciences, Bedford, MA

INTRODUCTION

A complex set of biophysical (e.g., topography, stiffness, viscosity, porosity) and biochemical (e.g., nutrients, matrix composition, cell-matrix interactions) cues control cell biology[1]. In order to mimic the *in vivo* cellular behavior for understanding basic biology, tissue engineering, and regenerative medicine applications, it is imperative to better elucidate and control these interactions. The importance of matrix elasticity is being increasingly studied and the stiffness (or elastic modulus) of naturally derived, as well as engineered hydrogel substrates has been shown to direct stem cell differentiation[2], effect cell migration[3], modulate force of muscle contraction[4], and influence cell spreading and adhesion[5]. Interestingly, stiffness of tissues in abnormal conditions can also vary: normal rat liver tissue (0.3 to 0.6kPa) compared to cirrhotic liver (3 to 12 kPa)[6,7], and normal breast tissue (1.2kPa) compared to breast tumors (2.4 to 4.8kPa)[8]. This phenomenon may be causative and not necessarily only an outcome of a disease state[9].

Lineage specific differentiation of human mesenchymal stem cells (hMSCs) has been shown to vary with matrix stiffness, where hMSCs were directed into particular phenotypes: neurons (matrix stiffness of 0.1 to 1kPa), myoblasts (8 to 17kPa), and osteoblasts (25 to 40kPa)[2]. As rigidity of the cross-linked polyacrylamide gels increased, cells increased their secretory response, e.g., Collagen I. After several weeks of culture and based on matrix rigidity, the specific lineage of the cells was committed. In another study, liver cell differentiation into abnormal phenotypes (myofibroblasts) was driven both by soluble factors and increasing matrix stiffness[6,7]. Following this change, an increased amount of abnormal extracellular matrix (ECM) was produced, resulting in fibrosis (scarring).

Human prostate carcinoma tumor cell (DU-145 cell) migration speed has been influenced by increasing Corning Matrigel matrix stiffness, and the maximum speed was observed at intermediate stiffness[3]. Other factors found to affect migration speed included cell adhesion and proteolytic activity of the cells. Additionally, pore size within the matrix decreased as Matrigel matrix concentration increased; however, this only played a minor role in migration speed.

Increasing Matrigel matrix concentration (from 10% to 40%) was also demonstrated to increase the contraction force of engineered skeletal muscle constructs[4]. Cell survival and function were affected minimally by matrix composition.

Extracellular Matrices

Basement membrane is a thin layer of ECM proteins underlying all tissues *in vivo*[10]. Corning Matrigel matrix is a solubilized basement membrane preparation extracted from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma, a tumor rich in extracellular matrix proteins, including Laminin (a major component), Collagen IV, heparin sulfate proteoglycans, entactin/nidogen, and a number of growth factors that are found in normal EHS tumors[11,12]. Matrigel matrix has been used in a wide range of applications such as stem cell culture, cell attachment and differentiation, angiogenesis assays, tumor growth, and tissue engineering[13,14].

Collagen is the most abundant protein present in animals, providing structural and mechanical support[15,16]. There are many different types of Collagen (about 28 known to date)[15] and it is

most plentiful in dermis, tendon, and bones[14]. The type I molecule of Collagen is a heterotrimer of 300nm length and is composed of two alpha$_1$(I) chains and one alpha$_2$(I) chain.

Corning Collagen I rat tail is prepared from rat tail tendons by acid extraction[17]. No enzymes are required for digestion, thus the telopeptides are preserved. Collagen can be used in a thin layer to promote cell attachment, and study tumor cell invasion and migration, fibrillogenesis, as well as to culture and differentiate monocytes or macrophages and to maintain hepatocyte function18. Collagen I can also be used in 3D gel form or electrospun into membranes to support 3D cell growth and differentiation[14].

Mechanical Properties of ECMs

Mechanical properties such as stiffness or elastic modulus of materials can be measured using a variety of techniques such as bulk compressive/tensile testing, rheometry, dynamic light scattering, atomic force microscopy (AFM), and nanoindentation[19,20]. In rotational or dynamic rheometry, viscoelastic material properties are measured by applying oscillatory shear strain on the sample, most commonly performed in a parallel-plate set-up. For example, in one study, different Corning Matrigel matrix (standard product) concentrations ranging from 50% to 100% were tested using a parallel-plate rheometer, and the stiffness (G') linearly increased from approximately 10 to 50Pa22. In a different report, storage modulus measured using a stress-controlled rheometer for Matrigel matrix (high concentration product) concentrations of 4.4, 8, and 17mg/mL was approximately 20, 70, and 300Pa, respectively[21]. A stiffness value of 44Pa has been reported for Matrigel matrix (high concentration product)[22]. Similarly, several publications have reported the stiffness values for Collagen I gels. Using a rotational rheometer, stiffness of Collagen I (rat tail, 2mg/mL) was reported to be 9 Pa[23]. Furthermore, when Collagen I was mixed with Matrigel matrix at a concentration of either 2 or 4mg/mL, the stiffness increased to 13.4 and 40.7 Pa, respectively. In another report, the stiffness of Collagen I gels (rat tail and porcine) in the concentration range of 0.5 to 4mg/mL increased from 0.5 to 100Pa[24].

Different mechanical responses are also observed when compressive forces are used instead of shear forces. Using displacement control unconfined compressive testing methodology, a modulus of 6kPa was achieved for Collagen I (2mg/mL) samples[25]. The constructs became increasingly stiffer with the addition of Matrigel matrix (10% to 50% addition) with moduli ranging up to 10kPa. The values obtained in this study are orders of magnitude higher than those from rotational rheometry studies.

Localized mechanical properties can be measured using AFM indendation by pressing a sharp tip into the sample. Under physiological conditions (37°C and aqueous), the average elastic modulus of Matrigel matrix (standard product) measured using AFM with a micron-sized spherical tip was 443Pa[26]. The differences in the stiffness results between rheological and AFM measurements could be due to differences in length scales (bulk vs. surface) in the two methods.

It should be noted that most of the cell culture work is typically performed on 2D substrates made of rigid materials such as glass and polystyrene, sometimes coated with a thin layer of ECMs. For example, polystyrene and glass substrates have a stiffness value in the GPa range which is orders of magnitude higher than that for ECM proteins (Pa to kPa range) causing the cells to show a non-natural behavior[1].

At the same time, results from measurements of mechanical properties of the thin ECM layers might be confounded by the bulk substrate stiffness.

In this paper, we examined the elastic moduli of the Matrigel matrix and Collagen I gels as a function of the protein concentration. The rheological measurements were performed using single frequency temperature sweeps on a rotational rheometer.

MATERIALS AND METHOD

Matrigel Preparation

Corning Matrigel Growth Factor Reduced (Matrigel GFR, VWR Cat. No. 47743-720) and Corning Matrigel High Concentration, Growth Factor Reduced (Matrigel HC GFR, VWR Cat. No. 80094-330) were thawed overnight in ice as directed in the Guidelines for Use[27-29]. The Matrigel matrix products were diluted to the desired protein concentrations using the lot-specific protein concentrations provided on the certificates of analysis using ice-cold Dulbecco's Modified Eagle Medium (DMEM). The contents of the vials were mixed by pipetting up and down and the calculated amount of Matrigel matrix was pipetted using a positive displacement pipet, to ensure accuracy, and added to ice-cold DMEM in pre-chilled glass tubes held in ice. The diluted samples were vortexed gently and held in ice. As polymerization of Matrigel matrix is temperature-dependent

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TIPS FOR SUCCESSFUL USAGE OF CORNING® MATRIGEL MATRIX®

Storage
- Store in a -20°C non-frost-free freezer.
- Minimize temperature fluctuations, do not store on the door or in a freezer that is opened frequently.

Thawing
- Pack in ice (completely submerge).
- Cover ice bucket and place in 2°C to 8°C location (cold room or refrigerator) overnight.
- Avoid repeated freeze thaw cycles.
- Thaw one-time use aliquots using the same guidelines, time may be adjusted based on the volume of the aliquot.

Diluting
- Use lot-specific protein concentration provided on the Certificate of Analysis to prepare dilutions.
- Keep Matrigel matrix and all solutions on ice.
- Add Matrigel matrix to ice-cold solutions (do not dilute with water, aggregates may form).
- Use pre-chilled tips, tubes, and cultureware.
- Use positive displacement pipets for accurate pipetting (due to viscosity).

Handling
- Plan ahead.
- Thaw Matrigel matrix appropriately.
- Make one-time use aliquots.
- Prepare Matrigel matrix aliquots immediately after thawing the vial.
- Store one-time use aliquots using the same recommended storage conditions.
- Aliquot non-diluted Matrigel matrix only.
- Matrigel matrix is temperature-sensitive and will start to gel above 10°C.
- Polymerization is non-reversible.

TIPS FOR SUCCESSFUL USAGE OF CORNING COLLAGEN I, RAT TAIL

Storage
- Store at 2°C to 8°C.
- Do not freeze the product.

Diluting
- Use lot-specific protein concentration provided on the Certificate of Analysis to prepare dilutions.
- Use ice-cold solutions.
- Keep product and diluted samples on ice.
- Use positive displacement pipets for accurate pipetting (due to viscosity).

Handling
- Polymerization is pH- and temperature-dependent.
- Collagen must be neutralized in order to form a gel.

$$\frac{\text{Desired final volume (mL)} \times \text{Desired protein concentration (mg/mL)}}{\text{Lot-specific protein concentration (mg/mL)}} = \text{volume of Matrigel matrix required (mL)}$$

Desired final volume (mL) - volume of Matrigel matrix required (mL) = volume of ice-cold diluent required (mL)

For example, to prepare a 10mL solution at 9 mg/mL using Corning Matrigel HC GFR (Lot No. 7002569) released with a protein concentration of 19.1mg/mL:

10 mL x 9 mg/mL
19.1 mg/mL = 4.7 mL

For example, to prepare a 5 mL solution at 9mg/mL using Matrigel GFR (Lot No. 7023006) released with a protein concentration of 9.5 mg/mL:

5 mL x 9 mg/mL
9.5 mg/mL = 4.74 mL

and non-reversible, it is important to prepare and hold the samples in ice. The diluted Matrigel matrix samples were mixed by inversion immediately before loading the test samples onto the rheometer using a positive displacement pipet (0.680mL). Each protein concentration was measured three times. Samples with concentrations of 3, 6, 9, and 15mg/mL were made using Matrigel HC GFR. A sample with a concentration of 9mg/mL was made using Matrigel GFR. The volumes of Matrigel matrix and ice-cold DMEM diluent required were calculated as follows:

Collagen I Preparation

Corning Collagen I High Concentration, rat tail (Collagen HC rat tail, VWR Cat. No. 47747-218) gelation is pH- and temperature-dependent, and the product must be neutralized in order to polymerize. Collagen HC rat tail was diluted using the alternate gelation procedure included in the lot-specific Certificate of Analysis[30,31]. All dilutions were prepared on ice in pre-chilled glass tubes.

To prepare the desired volume and protein concentration of Collagen I solutions, 0.1X the final volume of 10X Dulbecco's

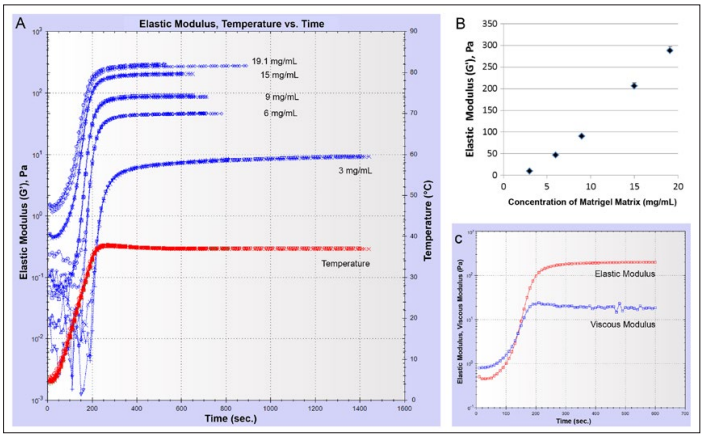

Figure 1. Increase of elastic modulus as a function of Corning Matrigel HC GFR concentration. (A) A plot of the elastic modulus (G') as a function of time for samples of Matrigel matrix at different concentrations. Temperature was ramped from 5°C to 37°C and held at 37°C. (B) Plateau values of G' (in Pa) shown as a function of Matrigel matrix concentration (n = 3). (C) Representative time course of elastic modulus and viscous modulus for Matrigel matrix at a concentration of 15mg/mL.

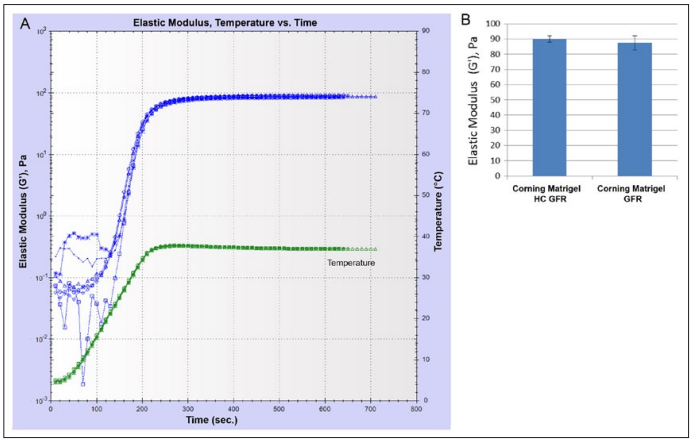

Figure 2. Comparison of elastic modulus (G') for two different Corning Matrigel matrix products formed at the same protein concentration (9 mg/mL). (A) A plot of the elastic modulus as a function of time for samples of Matrigel HC GFR and Matrigel GFR. Temperature was ramped from 5°C to 37°C and held at 37°C. (B) Plateau values of G' (in Pa) shown for the two different Matrigel matrix products (n = 3).

Phosphate Buffered Saline (10X DPBS), 0.023mL of 1N Sodium Hydroxid per mL of Collagen I were added and the final volume was adjusted using ice-cold DI-water. The contents of the tube were mixed by vortexing and the required volume of Collagen I was added to the solution using a positive displacement pipet, mixed by vortexing gently and held on ice. All diluted samples were mixed by inversion immediately before loading the test samples onto the rheometer using a positive displacement pipet (0.680mL). Each protein concentration was measured in triplicate.

Samples with concentrations of 1, 3, and 7mg/mL were made using Collagen HC rat tail.

For example, to prepare a 6 mL solution with a final Collagen I concentration of 7 mg/mL using Collagen HC rat tail (Lot No. 7016285) with a protein concentration of 9.82mg/mL:
0.6mL of 10X DPBS, 0.0984mL of 1N NaOH and 1.02mL of ice-cold DI-water were added to a pre-chilled glass tube and mixed by vortexing. Using a positive displacement pipet, 4.28mL of Collagen HC rat tail was added to the glass tube.

Rheological Measurements

The samples were analyzed using the Kinexus® Ultra+ Rotational Rheometer (Malvern Instruments) using a parallel plate (40mm roughened) configuration. Using a positive displacement pipet, samples (0.680mL) were directly added to center of the lower plate at a temperature of 5°C. The top plate was immediately lowered before the gel started to form, to a working gap of 0.5mm. The temperature was ramped from 5°C to 37°C at a rate of 10°C/minute and the temperature was held at 37°C. A solvent trap was used to cover the sample and prevent any evaporation effects around the perimeter. Viscoelastic measurements were performed periodically at a single frequency of 0.5Hz and 0.2% strain. Measurements were recorded until the

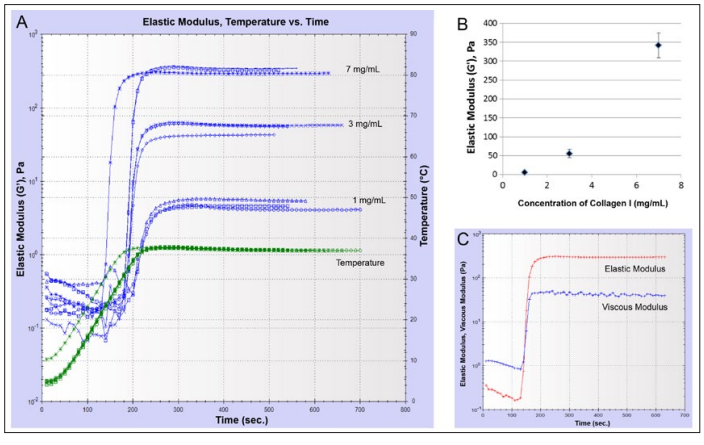

Figure 3. Increase of elastic modulus as a function of Collagen HC rat tail concentration. (A) A plot of the elastic modulus (G′) over time for samples of Collagen I at different concentrations. Temperature was ramped from 5°C to 37°C and held at 37°C. (B) Plateau values of the shear modulus (elastic component, G′) (Pa) vs. Collagen I concentration (n = 3). (C) Representative time course of elastic modulus and viscous modulus for Collagen I at a concentration of 7mg/mL.

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elastic modulus reached equilibrium. The data was acquired and analyzed using the rSpace® software (Malvern Instruments).

RESULTS AND DISCUSSION

Proteins present in naturally derived ECMs such as Corning Matrigel matrix and Collagen I self-assemble into complex 3D fibrous structures[32]. Mechanical properties of these matrices regulate a myriad of cellular functions including cell spreading, proliferation, migration, and differentiation[11]. Mechanical properties such as stiffness or elastic modulus of materials can be measured using a variety of techniques such as bulk compressive/tensile testing, rheometry, AFM, and nanoindentation.

To investigate whether varying the protein concentration alters the elastic modulus of the Matrigel matrix gels, Corning Matrigel High Concentration, Growth Factor Reduced (Matrigel HC GFR; at a concentration of 19.1mg/mL) was diluted using ice-cold DMEM and liquid samples were directly added to the rotational rheometer. The temperature of the bottom plate (Peltier-controlled) was ramped from 5°C to 37°C and then held at 37°C, which is the recommended gelation temperature for Matrigel matrix. The evolution of the elastic component (G') and the viscous component (G") of the complex shear modulus were observed over time and the sample was allowed to reach a state of equilibrium. Over a range of concentrations from 3 to 19.1mg/mL for Matrigel HC GFR, the elastic component of the shear modulus increased from 9.1 ± 0.3Pa to 288.2 ± 9Pa (Figures 1A and 1B). At the equilibrium state, the plateau value of the elastic component was larger than the viscous component indicating that the gel finally formed was elastic in nature (Figure 1C).

REFERENCES

For a complete list of references, visit ***vwr.com/vwrbiomarke.***

Description	Size	Cat. No.	Unit
Corning® Matrigel® Matrix, Growth Factor Reduced (GFR)	10 mL	**47743-720**	**Each**
Corning Matrigel Matrix (High Concentration GFR)	10 mL	**80094-330**	**Each**
Collagen I, High Concentration, Rat Tail,	100 mg	**47747-218**	**Each**
Dulbecco's Phosphate-Buffered Saline (DPBS)	500 mL	**45000-426**	**Pack of 6**
Dulbecco's Modification of Eagle's Medium (DMEM)	500 mL	**45000-304**	**Pack of 6**

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AriaMx Real-Time PCR System	**76193-704**	**Each**
ATTO425 Optical Cartridge	**76193-732**	**Each**
CY3 Optical Cartridge	**76193-728**	**Each**
CY5 Optical Cartridge	**76193-730**	**Each**
HEX Optical Cartridge	**76193-726**	**Each**
ROX Optical Cartridge	**76193-724**	**Each**
SYBR/FAM Optical Cartridge	**76193-706**	**Each**

SureVector Cloning and Protein Expression

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Abstract

SureVector is a state-of-the-art NextGen cloning system to rapidly design and build your own custom plasmids. The SureVector cloning method allows multiple DNA modules to be combined together into a recombinant plasmid that contains your target gene of interest (GOI) and any additional features you want to express. Our main goal was to clone and overexpress a protein of interest. First, we used the SureVector system to create a plasmid containing our gene of interest (pSV). Second, we transformed the plasmid into *E. coli* BL21(DE3) cells, and produced our recombinant protein of interest fused to a 6xHis tag. Finally, we purified our protein of interest with a His-Trap column. In addition, we were also interested in removing the 6xHis tag. To achieve this goal, we quickly created another plasmid, SureVector-Thrombin (pSVT) using the SureVector system. To do this, we introduced a thrombin cleavage site between the 6xHis sequence and the gene of interest by modifying one of the original PCR primers, to enable us to enzymatically remove the 6xHis tag after protein purification. Our plasmids were created with the following SureVector DNA modules: a promoter-tag fusion (T7-His6), a selection marker (Kanamycin resistance), an origin of replication (pUC), an XP1 fragment (XP1), an XP2 fragment (lactose inhibitor) and our gene of interest.

Gene Amplification with Overlapping Sequence

The gene of interest was amplified by PCR to append the appropriate overlapping sequences of the adjacent DNA modules. We performed a PCR amplification with the designed downstream and upstream primers, which included 30 nucleotides of overlapping sequence as recommended by the SureVector User Manual, and an additional 16 nucleotides of the gene of interest (Table 1). The first three nucleotides of the upstream primer that complement the gene of interest encode the first amino acid codon for the gene of interest. In the downstream primer, the end of the region that complements the gene of interest includes a stop codon.

SureVector (pSV)
Upstream primer 5' GGTGGCGGAGGTTCTGGAGGCGGTGGAAGT-GOI 3'
Downstream primer 5' CTCGAGGAGATATTGTACACTAAACCAAATG-GOI 3'

SureVectorThrombin modified (pSVT)
Upstream primer 5' GGTGGCGGAGGTTCTGGCCTGGTGCCGCGCGGCAGC-GOI 3'
Downstream primer 5' CTCGAGGAGATATTGTACACTAAACCAAATGC-GOI 3'

Table 1. Gene of interest PCR Primer Overlap

In order to create a second unique plasmid containing a thrombin cleavage site, we designed a different upstream primer, replacing the last 13 nucleotides of the 30 nucleotides of SureVector overlapping sequence that codify glycine residues with the thrombin cleavage site sequence (CTGGTGCCGCGCGGCAGC), while keeping the remaining 17 nucleotides overlapping the gene of interest (Table 1).

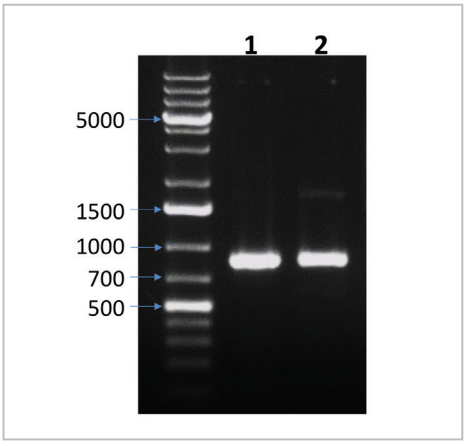

Figure 1. Agarose gel analysis of PCR amplification products. Lane 0: molecular weight markers; lane 1: PCR product of the GOI for pSV; lane 2: PCR product of the GOI with thrombin cleavage site for pSVT.

Thus, we performed two different PCR reactions: one to amplify the gene of interest with overlapping sequences that the SureVector manual recommended (1), and a second to amplify the gene of interest with a thrombin cleavage site (2). The PCR product was purified and the DNA checked for the correct size using an agarose gel (Fig.1).

Assembly Reaction and Transformation

We next carried out the SureVector assembly reaction, creating our two plasmids: pSV and pSVT. We followed the SureVector protocol as recommended in the User Manual, using a thermal cycler. Each reaction was incubated with DpnI for 5 minutes at 37ºC. Subsequently, we performed transformation reactions with 45µL of XL10-Gold Supercompetent Cells, and plated samples of each transformation onto LB-agar plates containing kanamycin, which were then incubated at 37ºC overnight.

After 16 hours, we examined the plates for colonies. A lot of colonies grew on the pSV plate (Fig. 2), and a smaller number grew on the pSVT plate where the gene of interest included the thrombin cleavage site (Fig.3). One possible reason for this could be the decreased length of overlapping sequence in the upstream primer. In the future, higher amounts of DNA could be used in the assembly reaction in order to get more colonies.

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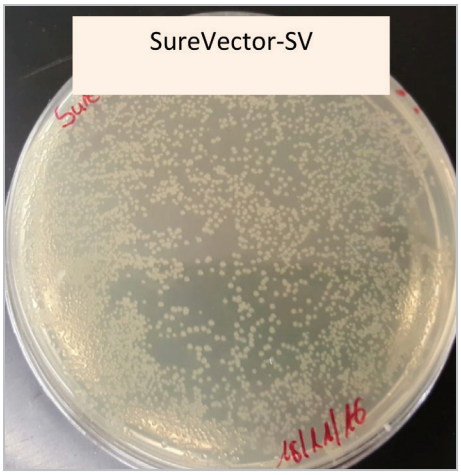

Figure 2. Transformation plate containing pSV (SureVector with gene of interest) colonies.

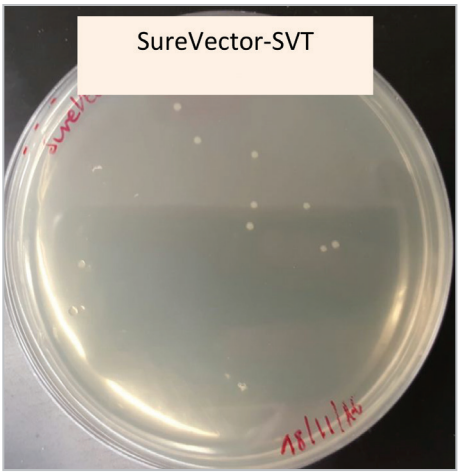

Figure 3. Transformation plate containing pSVT (SureVector with gene of interest and thrombin cleavage site) colonies.

Nevertheless, the colonies obtained in this experiment were all positive.

We next randomly selected colonies from each assembly for testing and verification by restriction digest. Three single colonies from the pSV plate and another three single colonies from the pSVT plate were picked and expanded in 5mL of LB medium supplemented with 100ng/µL of Kanamycin. Plasmid DNA was purified by miniprep, and 100ng/µL of each sample,

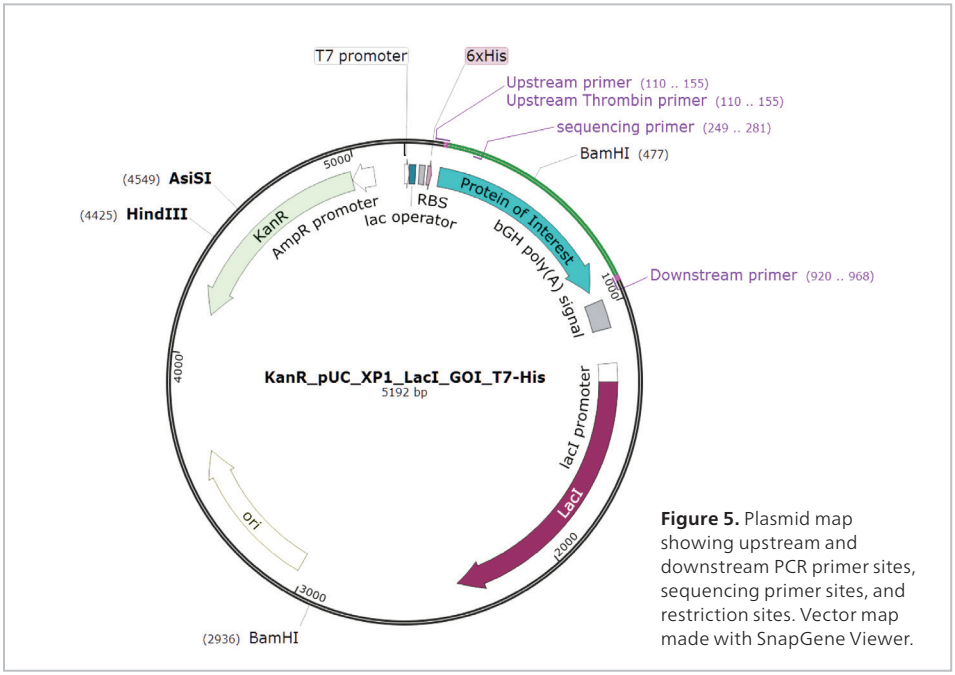

Figure 5. Plasmid map showing upstream and downstream PCR primer sites, sequencing primer sites, and restriction sites. Vector map made with SnapGene Viewer.

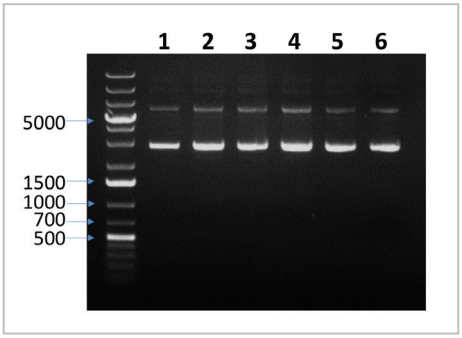

Figure 4. Agarose gel analysis of plasmids. Lane 0: molecular weight markers; lane 1: pSV-1; lane 2: pSV-2; lane 3: pSV-3; lane 4: pSVT-1; lane 5: pSVT-2; lane 6: pSVT-3.

SureVector plasmid (pSV) and SureVector Thrombin plasmid (pSVT), were separated on a 0.7% agarose gel to check their quality (Fig.4).

Plasmid Verification

To verify that each vector contained our gene of interest, plasmid DNA was cut with a single site restriction enzyme, AsiSI. The anticipated vector digest would result in a single band of 5198bp. A second restriction analysis was performed using two restriction enzymes simultaneously, BamHI and HindIII. This would result in three bands of 1250bp, 1489bp, and 2459bp. We used both BamHI and HindIII because BamHI cuts inside our gene of interest and HindIII cuts in the vector backbone, generating 3 specific DNA fragments (Fig. 5). We ran 200ng of each DNA product on an agarose gel, and the sizes of the fragments obtained were as expected (Fig. 6). Therefore, all six DNA plasmids were correct and the efficiency of the system was very good. Lastly, pSV-3 and pSVT-3 composition were further confirmed by Sanger DNA sequencing.

Protein Expression

Both plasmids, pSV and pSVT, were transformed into *E.coli* BL21(DE3) cells to

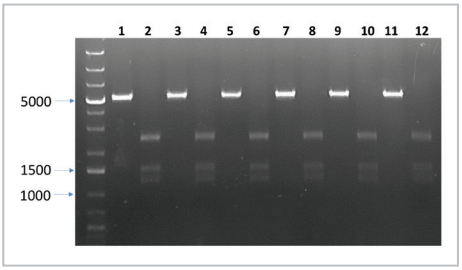

Figure 6. Agarose gel analysis of plasmids digested with restriction enzymes. Lane 0: molecular weight markers; Lane 1: pSV-1 digested with AsiSI; lane 2: pSV-1 digested with BamHI and HindIII; lane 3: pSV-2 digested with AsiSI; lane 4: pSV-2 digested with BamHI and HindIII; lane 5: pSV-3 digested with AsiSI; lane 6: pSV-3 digested with BamHI and HindIII; lane 7: pSVT-1 digested with AsiSI; lane 8: pSVT-1 digested with BamHI and HindIII; lane 9: pSVT-2 digested with AsiSI; lane 10: pSVT-2 digested with BamHI and HindIII; lane 11: pSVT-3 digested with AsiSI; lane 12: pSVT-3 digested with BamHI and HindIII.

express the protein of interest. The expression of the protein of interest is controlled by IPTG induction, and the pilot assay included further purification using Ni-NTA, a nickel-charged affinity resin that specifically recognizes the 6XHis-tag. Transformed cells were grown in 100mL LB medium at 37ºC with 180 rpm shaking. When the OD = 0.8, the cells were induced with IPTG (final concentration 0.5mM) and the cells further incubated at 25ºC and 140 rpm for 6 hours. To check the production of protein, samples were taken at 0 hours (control, no IPTG induction), 2 hours, 4 hours, and 6 hours (20mL of cells were harvested by centrifugation at 4500 rpm for 15 minutes and washed with PBS). These cells were resuspended in lysis buffer containing protease inhibitors (10mM benzamidine, 1mM PMSF, 10µg/mL trypsin inhibitor). The cells were lysed and centrifuged at 14,000 rpm for 30 min at 4ºC. The supernatants were incubated with Ni-NTA resin, and the resin was washed two times. Finally, the bound protein was eluted with elution buffer containing 500mM imidazole. Our protein of interest with the thrombin cleavage site was further processed by a 1h thrombin incubation to remove the 6xHis tag. The expected sizes of the target proteins are 32.51kDa with the 6xHis tag, and 30.85kDa after thrombin cleavage to remove the 6xHis tag. Equal amounts of each protein sample was loaded onto a 17% SDS-PAGE. Our results show a highly purified protein of the expected molecular weight, with maximal expression 6h after IPTG induction (Fig. 7). Furthermore, the pSVT plasmid containing the thrombin cleavage site was shown to work very well, as the amount of protein was slightly higher and the tag can be completely removed with thrombin incubation (Fig. 8).

Conclusions

We can conclude that the SureVector system is a good choice to make your own custom plasmids in an easy and quick way. Furthermore, it can be adapted to individual needs as demonstrated by the inclusion of a thrombin cleavage site in one of our vector assemblies. In addition, we have obtained a significant amount of our protein of interest enabling us to directly continue our research.

Description	Cat. No.	Unit
SureVector Core Kit	76193-662	Each
SureVector E. coli Selection Kit	76193-676	Each
SureVector E. coli N-Terminal Tag Kit	76193-682	Each
BL21 (De3) Competent Cells, 5 x 0.2 mL	99900-000	Each
XL10-Gold Ultracompetent Cells, 10 x 100 µL	99900-018	Each

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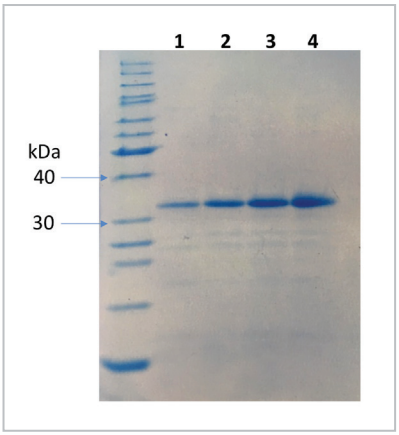

Figure 7. SDS-PAGE analysis of pSV protein expression. Lane 0: molecular weight markers; lane1: pSV-3 (Control, 0 hours IPTG induction); lane 2: pSV-3 (2 hours IPTG induction); lane 3: pSV-3 (4 hours IPTG induction); lane 4: pSV-3 (6 hours IPTG induction). Note that the control (no IPTG, time 0 hours) normally expresses a small amount of the protein of interest due to the high basal activity levels of the T7 RNA polymerase of the bacterial strain BL21(DE3). Expected size of target protein: 32.51kDa with 6xHis tag.

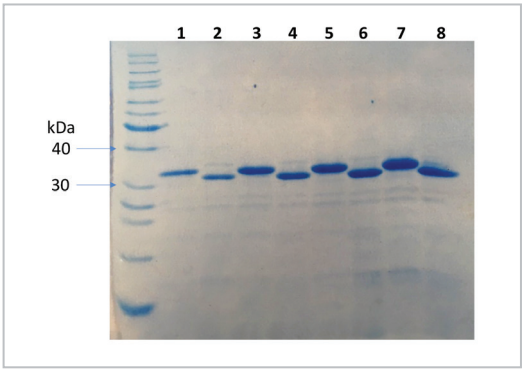

Figure 8. SDS-PAGE analysis of pSVT protein expression. Lane 0: molecular weight markers; lane1: pSVT-3 (Control, 0 hours IPTG induction, before thrombin cleavage); lane 2: pSVT-3 (Control, 0 hours IPTG induction, after thrombin cleavage); lane 3: pSVT-3 (2 hours IPTG induction, before thrombin cleavage); lane 4: pSVT-3 (2 hours IPTG induction, after thrombin cleavage); lane 5: pSVT-3 (4 hours IPTG induction, before thrombin cleavage); lane 6: pSVT-3 (4 hours IPTG induction, after thrombin cleavage); lane 7: pSVT-3 (6 hours IPTG induction, before thrombin cleavage); lane 8: pSVT-3 (6 hours IPTG induction, after thrombin cleavage). Note that the control (no IPTG, time 0 hours) normally expresses a small amount of the protein of interest due to the high basal activity levels of the T7 RNA polymerase of the bacterial strain BL21(DE3). Expected sizes of target proteins: 32.51kDa with 6xHis tag, 30.85kDa without 6xHis tag after thrombin cleavage.

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Description	Size	Cat. No.	Unit
Monarch Total RNA Miniprep Kit	50 Preps	103529-148	Each
Monarch RNA Purification Columns	100 Columns	103529-146	Each
Monarch gDNA Removal Columns	100 Columns	103529-158	Each
Monarch Collection Tubes II	100 Tubes	103529-160	Each
Monarch DNA/RNA Protection Reagent	56 mL	103529-150	Each
Monarch RNA Lysis Buffer	100 mL	103529-152	Each
Monarch RNA Priming Buffer	56 mL	103529-154	Each
Monarch RNA Wash Buffer	50 mL	103529-156	Each
Monarch Total RNA Miniprep Enzyme Pack	—	103529-162	Each

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Description	Reaction Size	Cat. No.	Unit
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NEBNext Ultra II Directional RNA Library Prep Kit for Illumina	96	103501-462	Each
NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads	24	103501-468	Each
NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads	96	103501-466	Each
NEBNext Ultra II RNA Library Prep Kit for Illumina	24	103501-446	Each
NEBNext Ultra II RNA Library Prep Kit for Illumina	96	103501-444	Each
NEBNext Ultra II RNA Library Prep with Sample Purification Beads	24	103501-456	Each
NEBNext Ultra II RNA Library Prep with Sample Purification Beads	96	103501-454	Each

Enhanced CRISPR/Cas9 Genome Editing Using RNP Delivery with jetCRISPR™

By Alengo Nyamay'Antu, Fanny Prémartin, Thibaut Benchimol, Valérie Toussaint, Patrick Erbacher, Polyplus-transfection

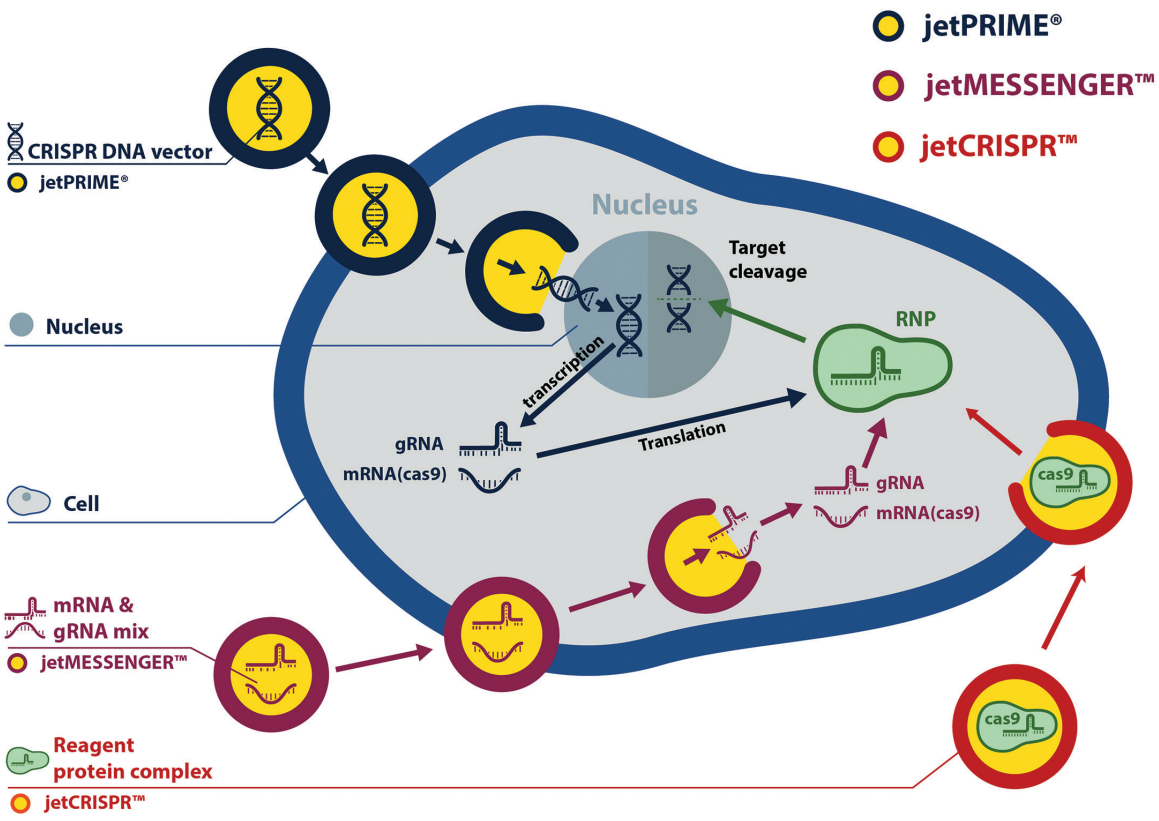

Fig. 1: *in vitro* transfection approaches for CRISPR experiments.

Introduction

The association of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) nucleases is an innovative technology used to generate gene knock-outs, introduce defined sequences, or select deletions in the genome. The CRISPR/Cas9 engineered nuclease system is a two-component system with a guide RNA (gRNA) molecule that drives the Cas9 nuclease to create a double-strand break at a specific targeted sequence within the genome.

Successful targeted delivery of the gRNA and the Cas9 into cells is indispensable to guarantee the high genome editing efficiency that is required to generate new cell or animal models. Transfection is therefore a key and often limiting step to ensure successful genome editing. There are currently three main approaches to introduce gRNA and Cas9 in mammalian cells: DNA, RNA, or RNP (gRNA + Cas9 protein) (Fig. 1).

Each approach has its own pros and cons in terms of easiness of use, genome editing efficiency, and off-targeting effects. Delivery of both gRNA and Cas9 as plasmid DNA is the go-to approach because synthesis and delivery of plasmid DNA is commonly used, and is often easier to implement for a first genome editing approach. While plasmid DNA leads to high genome editing in easy to transfect cells and can facilitate the generation of pure, stable, mutant cell lines, it is less adapted for difficult to transfect

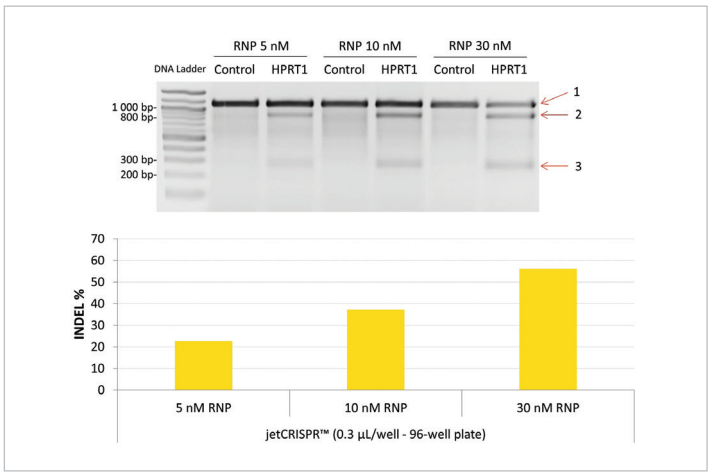

Fig. 2: High genome editing efficiency using jetCRISPR™ in HeLa cells. RNP transfections were performed in HeLa cells using several RNP concentrations of Cas9 protein and HPRT1 gRNA or negative control in combination with 0.3µL of jetCRISPR™ reagent per well of a 96-well plate. At 48 h post-transfection, T7 digestion products were run on a 2% agarose gel and stained with BET displayed by G:Box transilluminator (Syngene®). Acquisitions were carried out with the Genesnap software (Syngene®) and INDEL quantifications were performed with the Genetools software (Syngene®). 1: Uncleaved fragment of 1083bp, 2: long cleaved fragment of 827bp, 3: short cleaved fragment of 256bp.

primary cells and cancer cell lines, notably due to their slow to non-dividing rate. In addition, delivery of the Cas9 protein as plasmid DNA will lead to a more prolonged expression of the Cas9 protein, which can result in a higher amount of off-target cleavage activity.

DNA-free delivery systems in which Cas9 is delivered as mRNA or as a protein have thus become attractive alternatives, as they overcome the main obstacle that DNA entry into the nucleus constitutes in harder to transfect cells. In contrast to a plasmid DNA approach, delivery of Cas9 as mRNA, or even more so as a protein leads to a more transient and time-restricted presence of Cas9 within cells before being rapidly cleared via the protein degradation pathways. Consequently, delivery of Cas9 as a protein, is currently the best-suited approach to reduce potential off-target nuclease activity when specificity is a main concern.

Here, we present jetCRISPR, our latest innovative reagent designed to directly deliver Cas9 as a protein along with the guide RNA, also known as ribonucleoprotein (RNP) delivery. Both transcription and translation to obtain a functional Cas9 protein is bypassed, which makes RNP delivery using jetCRISPR™ the fastest and most precise gene editing approach currently available for a wide variety of cells.

High Genome Editing Efficiency Using RNP Transfection Approach

jetCRISPR™ transfection reagent is an innovative transfection reagent that efficiently delivers RNP complexes to reach highest levels of Cas9-mediated genome editing. During RNP transfection optimization, we carefully optimized gRNA and Cas9 protein concentration and ratio as well as the Cas9 protein sequence. Genome editing efficiency measured as the percentage of INDEL (%), was assessed within a range of RNP concentrations to target one of the frequently used housekeeping gene HPRT1 (Fig. 2). Several RNP concentrations were tested ranging from 5–30nM, with a gRNA to Cas9 ratio of 1:1. While transfection of RNP at the lowest range of 5nM generated 20% of INDEL in HeLa cells, we demonstrated that by increasing RNP concentration to 10nM, we could further increase by 2-fold editing of the targeted HPRT1 gene. Furthermore, with up to 30nM of RNP, 50% of INDEL was reached with minimal impact on cell viability and cell morphology.

The Cas9 protein sequence can also lead to significant variations in genome editing efficiency. RNP transfection optimization with commercially available recombinant Cas9 proteins was performed in HEK-293 cells to target one of the housekeeping genes, HPRT1. As shown in Fig. 3, the number of Nuclear Localization Sequence (NLS) and protein tags, as well as their positioning in N- or C-terminus could affect the genome editing efficiency. To this end, Polyplus-transfection also provides the Cas9 protein — SpCas9 nuclease — that led to the highest genome editing efficiency,

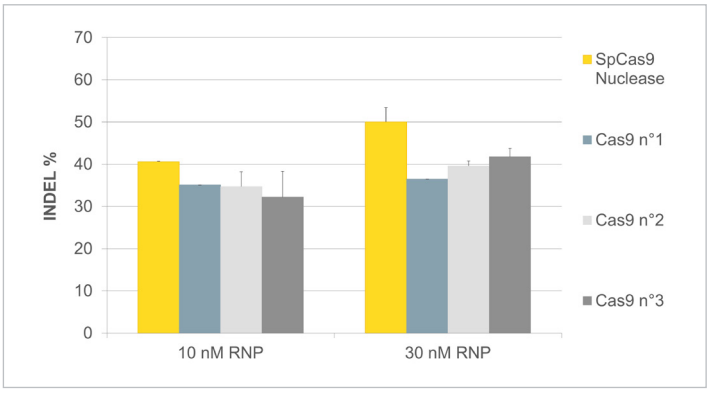

Fig. 3: Higher genome editing obtained with SpCas9 Nuclease compared to other Cas9 competitors using jetCRISPR™ transfection reagent. RNP transfections were performed in HEK-293 cells using 10 or 30nM RNP (HPRT1 sgRNA + Cas9 protein) with 0.3µL of jetCRISPR™ reagent per well of 96-well plate. At 48 h post-transfection, genome editing was assessed by calculating the percentage (%) of INDEL using the T7 endonuclease method. The INDEL % was determined by using Genetools software (Syngene®).

in vitro - in vivo	Delivered Molecule	Cas9	Guide RNA	Our Solutions
in vitro	Protein/RNA	Protein	RNA	jetCRISPR™ RNP transfection reagent
in vitro	DNA	DNA	DNA	jetPRIME® DNA transfection reagent
in vitro	RNA	mRNA	RNA	jetMESSENGER™ mRNA transfection reagent
in vitro	DNA/RNA	DNA/mRNA	DNA/RNA	*in vitro*-jetPEI® *in vitro* delivery reagent

Table 1: Polyplus-transfection's range of transfection reagents for CRISPR experiments.

irrespective of the concentration of RNP tested within the optimal range.

Superior Genome Editing Efficiency Obtained with jetCRISPR

RNP transfection using jetCRISPR is a straightforward 3-step process: assembling RNP complexes, mixing in of the transfection reagent and adding complexes to cells. Both reverse and forward transfections have been successfully optimized with jetCRISPR, hence facilitating the implementation of a protocol for a wide variety of cell types. In addition to its compatibility with different cell culture systems, jetCRISPR transfection reagent systematically outperforms its main competitor by achieving higher genome editing efficiency, as illustrated in Fig. 4.

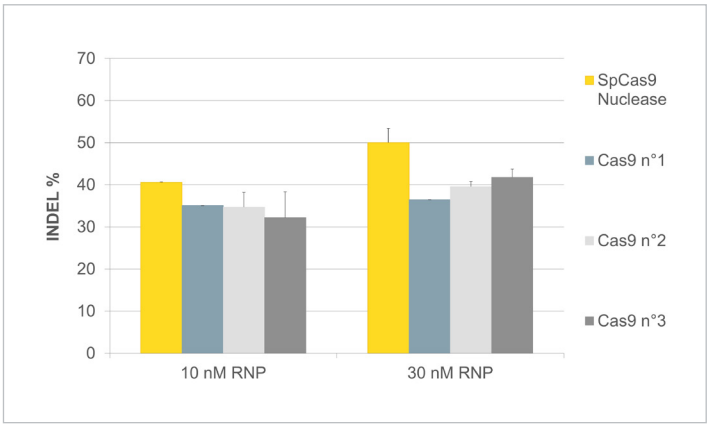

Fig.4: Superior genome editing efficiency obtained with jetCRISPR in comparison with Lipofectamine® CRISPRMAX™. RNP transfections were performed in A549 and HEK-293 cells using 30nM RNP (Cas9 Protein and HPRT1 gRNA) with 0.3µL of jetCRISPR reagent or 0.3µL of Lipofectamine CRISPRMAX, per well of a 96-well plate. At 48 h post-transfection, genome editing was assessed by calculating the percentage (%) of INDEL using the T7 endonuclease method. The INDEL % was determined by using Genetools software (Syngene®).

Conclusion

Successful delivery of the gRNA and the Cas9 into cells is essential to guarantee the high genome editing efficiency required to generate new cell or animal models. The most efficient CRISPR-Cas9 method is based on the direct delivery of pre-complexed gRNA and Cas9 protein, referred to as Ribonucleoprotein (RNP) delivery. RNP transfection has many advantages compared to plasmid or mRNA approaches, including the most important advantages are a better Cas9 activity control, a reduced amount of off-target effects, and the ability to maintain excellent cell viability.

With our recently launched RNP transfection reagent jetCRISPR™, we now provide a full range of reagents for all CRISPR approaches (Table 1). Use the leading technology for CRISPR/Cas9 RNP delivery!

- jetCRISPR for RNP delivery — Protein and guide RNA co-delivery
- jetPRIME® for CRISPR DNA plasmid approaches
- jetMESSENGER™ for RNA transfection (guide RNA and mRNA co-transfection)
- *in vivo*-jetPEI® for in vivo gene editing through delivery of DNA

References

For a complete list of references, visit ***vwr.com/vwrbiomarke.***

Description	Size	Cat. No.	Unit
jetCRISPR — RNP transfection reagent	0.1 mL	**76093-890**	**Each**
jetCRISPR — RNP transfection reagent	1.5 mL	**76093-894**	**Each**
jetMESSENGER — mRNA transfection reagent	0.75 mL	**75806-224**	**Each**
jetMESSENGER — mRNA transfection reagent	1.5 mL	**75806-226**	**Each**
jetPRIME — DNA transfection reagent	0.75 mL	**89129-922**	**Each**
jetPRIME — DNA transfection reagent	1.5 mL	**89129-924**	**Each**
jetPRIME — DNA transfection reagent	7.5 mL	**89129-926**	**Each**
SpCas9 nuclease	100 µg	**76196-696**	**Each**

Imaging Viral Load in Chicken Embryos

Introduction

Newcastle Disease Virus (NDV) is a contagious disease, capable of transmission to humans, and with a high risk of a severe epizootic in birds, particularly domestic poultry which are typically reared at high density.

While the effects of NDV are mild in humans, its effect in avian hosts is more pronounced and varies based on the age and species of the host, as well as the strain of the virus. Three strains of NDV, with differing levels of virulence, have been well characterized in chicken eggs. Strains of intermediary virulence are known to infect primarily the umbilical tissue of the chicken embryo, whilst more virulent strains infect other tissues indiscriminately.

As such, many studies have been performed to investigate the impacts of the varying strains along with the mechanisms of viral load in domestic chickens. However, such studies have often been limited in their ability to accurately and rapidly assess viral load *in vivo*. With the development of sensitive high-resolution imaging systems, such as the Azure c600, it is now possible to quickly and easily image fluorescently tagged NDV in chicken embryos, and, to some extent, quantify the viral load in various embryonic tissues.

Methods

Chicken embryos were infected at E10 and incubated for a further 72 hours. Embryos were infected with either 100–1000 PFU of virulent or non-virulent recombinant NDV tagged with eGFP, and compared with a noninfected control. The embryos were placed on Petri dishes and imaged with the Azure c600 using Epi Blue imaging mode with an automated capture.

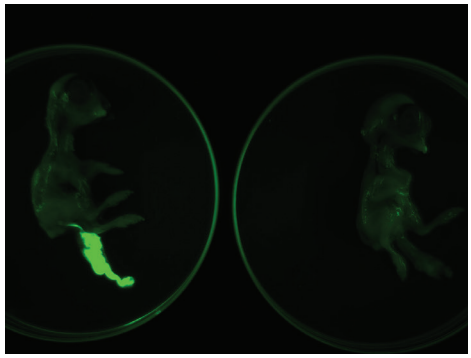

Figure 1. NDV eGFP No-VIR (left) and Negative Control (right).

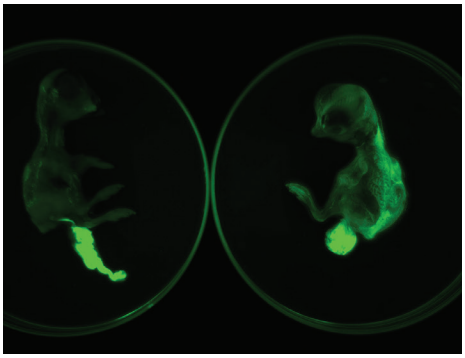

Figure 2. NDV eGFP No-VIR (left) and NDV eGFP VIR (right).

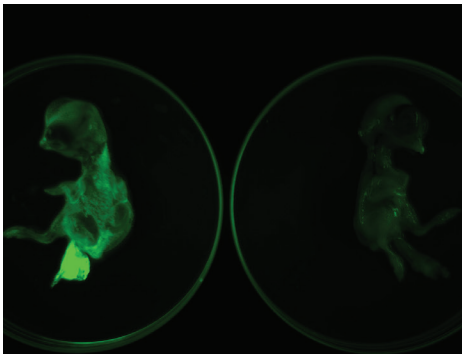

Figure 3. NDV eGFP VIR (left) and Negative Control (right).

Results

Figure 1 shows an embryo infected with a nonvirulent form of the virus next to an uninfected embryo (negative control). The infected embryo shows the non-virulent strain of the virus confined primarily in the umbilical tissue.

Figure 2 shows the same non-virulent virus-infected embryo imaged alongside an embryo infected with a more virulent strain of NDV. The virulent form of the virus infects the embryo more indiscriminately, and the virus is detected throughout the embryo.

Figure 3 shows the extent of the virulent infection compared to the negative control, uninfected embryo.

Conclusions

The utility of fluorescent imagers like the Azure Biosystems c600 extends far beyond Western blotting and includes a large number of applications that utilize fluorescence excitation. This application note showcases one such application – imaging a fluorescently tagged virus in animal models allowing for the generation of high-resolution quantifiable images.

Acknowledgements

Thank you to Ray Izquierdo-Lara, Ana Chumbe, Katherine Calderon, Manolo Fernandez. FARVET SAC, Peru, for providing the images.

Description	Cat. No.	Unit
The Ultimate Western Blot Imaging System, c600	10147-214	Each
The Infrared Imaging System, c500	10147-216	Each
The Visible Fluorescent Western Imaging System, c400	10147-218	Each
The Darkroom Replacer Imaging System, c300	10147-220	Each

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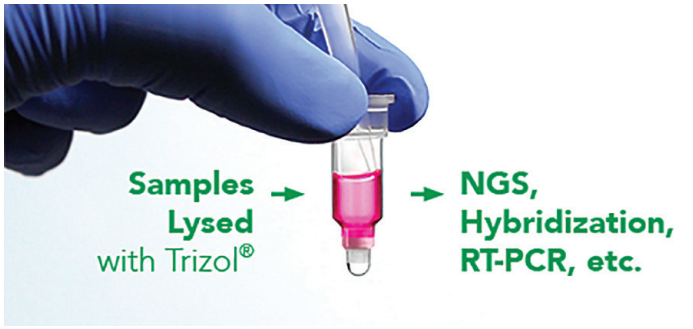

US ONLY

Description	Recovery	Cat. No.	Unit
Direct-zol RNA Microprep	10 µg	76020-456	Each
Direct-zol RNA Microprep with TRI Reagent	10 µg	76020-108	Each
Direct-zol RNA Miniprep	50 µg	76020-642	Each
Direct-zol RNA Miniprep with TRI Reagent	50 µg	76020-648	Each
Direct-zol RNA Miniprep Plus	100 µg	76020-110	Each
Direct-zol RNA Miniprep Plus with TRI Reagent	100 µg	76020-112	Each
Direct-zol-96 RNA	10 µg	76020-452	Each
Direct-zol-96 RNA with TRI Reagent	10 µg	76020-454	Each

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Description	Preps	Cat. No.	Unit
ZymoPURE II Plasmid Midiprep Kit	25	77001-396	Each
ZymoPURE II Plasmid Midiprep Kit	50	77001-466	Each
ZymoPURE II Plasmid Maxiprep Kit	10	77001-468	Each
ZymoPURE II Plasmid Maxiprep Kit	20	77001-470	Each
ZymoPURE II Plasmid Gigaprep Kit	5	77001-472	Each

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Description	Size	Cat. No.	Unit
Q 2-Channel qPCR Instrument	—	76175-392	Each
Q 4-Channel qPCR Instrument	—	76175-394	Each
Accessories			
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Description	Cat. No.	Unit
FlashGel DNA System	95045-604	Each
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DNA Cassette, 1.2%, 12+1 Well, Single Tier	95015-618	Pack of 9
DNA Cassette, 2.2%, 16+1 Well, Double Tier	95015-624	Pack of 9
RNA Cassette, 1.2%, 12 + 1 Well, Single Tier	95015-626	Pack of 9
Recovery Cassette, 1.2% Agarose, 8+1 (18-Well), Double Tier	95053-310	Pack of 9
DNA Marker (100 bp–4 kb), 500 µL	95015-632	Each
Loading Dye, 5 x 1 mL, 5X Concentrate	95015-630	Each

Real-Time PCR to Measure Minute Volumes in High Throughput

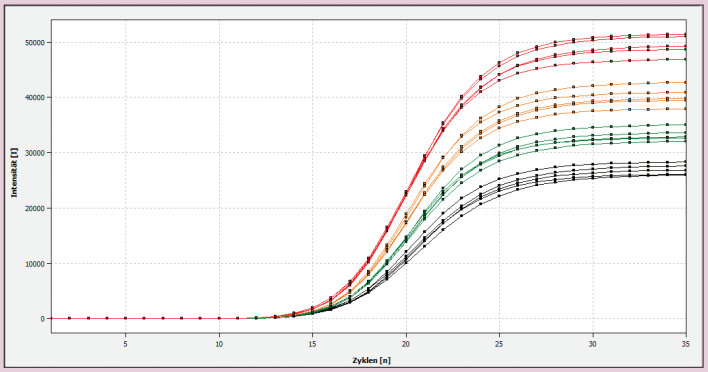

Figure 1a: Representation of the amplification of a 120 bp *E.coli*-specific target sequence in the qTOWER³ 84. In the image above, a manual reaction setup was used, using a manual pipette for the volume variation.

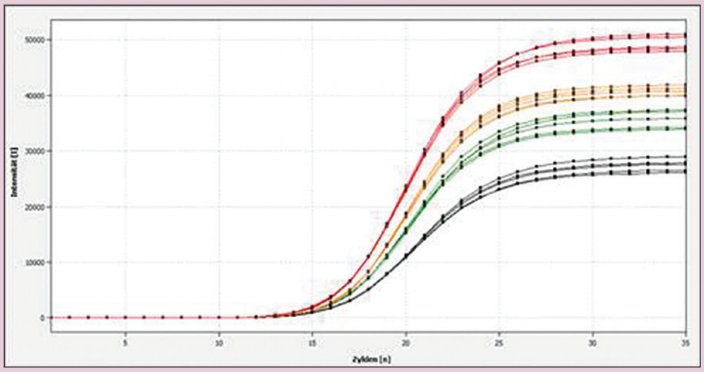

Figure 1b: Representation of the amplification of a 120bp *E.coli*-specific target sequence in the qTOWER³ 84. At this point, the qPCR was carried out with an automated reaction batch, produced using the GeneTheatre pipetting robot.

With the help of a pipetting robot and the standardization of the sample setups, substantiated and reproducible results can be achieved, especially in the 384-well high-throughput format — even in the smallest volume range.

Steadily-increasing sample volumes and new options for molecular biology experiments are changing the demands on quantitative real-time PCR. For these, the transition to the multi-well format with 384 samples opens up new application areas for gene expression, mutation analysis, and diagnostics.

Real-time PCR in 384-well high-throughput format requires a steady homogeneous readout of very low sample volumes, which, in addition to such factors as speed and experimental accuracy in the high-throughput format, will play an increasingly important role. One way that the qTOWER³ 84 from Analytik Jena provides this precise performance in 384-well high-throughput format is by means of an aluminum block with guaranteed temperature uniformity. Another way Analytik Jena delivers this is by expanding the Company's patented high-performance optics from the qTOWER³ product family to 16 fibers, thus enabling a readout time of just six seconds for a complete 384-well plate — regardless of the number of fluorescent dyes that are used.

The preparation effort required for performing a high-throughput qPCR is increasing. In addition, the setup for the reaction batch for qPCR requires certain skills and experience. Along with a quadrupling of the number of samples compared to the 96-well standard format, the reduced sample volume also plays an important role. These micro-volume ranges must be pipetted with extreme precision, because even the smallest variations in raw material can lead to major differences in amplification.

It is recommended to use a pipetting robot in order to standardize and improve the precision and reproducibility of real-time PCR reactions. This makes it infinitely easier to perform all pending pipetting tasks in the laboratory and enables full automation of PCR and real-time PCR reaction batches. The standardization of processes

A) Manual			B) Automated		
Reaction Volume	Ct	SD(Ct)	Reaction Volume	Ct	SD(Ct)
2 µL	14,926	0.05	2µL	14,896	0.03
5 µL	14,464	0.04	5 µL	14,430	0.02
10 µL	14,128	0.05	10 µL	14,168	0.02
20 µL	14,120	0.08	20 µL	14,096	0.04

Figure 3: Comparison of the automatically generated Ct values of the qPCRsoft in the qTOWER³ 84.

analytikjena

significantly reduces errors regarding reactions involving 10 or 5µL batch volumes — regardless of the experience and skill of the user. An automated reaction setup offers many advantages: utmost precision, for example, as well as the possibility to routinely process smaller reaction volumes than those which would be possible with manual batches.

Microbiology Application Example

Using DNA extracted from Escherichia coli, a sequence-specific primer pair and the double-concentrated innuMIX qPCR MasterMix SyGreen (Analytik Jena), an *E.coli*-specific target sequence of 120bp is amplified in real-time in the 384-well format.

In a variation of the reaction volume, five technical replicates of 5–20µL of master mix were used. Subsequently, the specificity of the amplification product was verified in a melting curve. The amplification is carried out in a FrameStar® qPCR plate (4titude) with an initial denaturing of 120s followed by 35 cycles, with denaturation at 95°C for 15s, annealing at 58°C for 15s, and elongation at 72°C for 30s. The fluorescence signal was recorded at 72°C in each cycle.

A manual reaction setup using a manual pipette was compared with an automated reaction batch that was pipetted using the GeneTheatre pipetting robot (Analytik Jena).

Two Microliters Are Sufficient

The results of the volume variation show that it is possible to reduce the reaction volume down to 2µL for this assay without any problems.

Even this small sample volume shows a standard deviation of the Ct values of only ± 0.05 over five replicates.

The amplification line plot from the automatically-generated reaction setup using the pipetting robot also shows a homogeneous distribution of the volume-varied real-time PCR curves. This shows that the standard deviation of the Ct values calculated by the software are again lower compared to the Ct values in the experiment with the manual pipette. This means that substantiated and reproducible results are achieved using a pipetting robot, especially in 384-well high-throughput format, through standardization of the sample setup and the pipetting routine — even in the smallest volume range.

qTOWER®³ 84 – The New Standard for qPCR

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Redefining Excellence with Precision and Speed

- **Efficient:** Ultrafast 6 second 384-well read out
- **Brilliant:** Optimal illumination of all 384 samples with no edge effect
- **Unrivaled:** Ideal temperature homogeneity and unmatched precision control (± 0.1°C)
- **Transparent:** No costs for software licenses or updates
- **Versatile:** Includes 24 column/zone gradient capability
- **Easy-to-Use:** No periodic maintenance or calibration required
- **Durable:** 10-year long-term guarantee for high performance optical components; 3-year warranty on the remainder of the system

Description	Cat. No.	Unit
qTOWER³ 84, Non-Gradient	76196-698	Each
qTOWER³ 84 G, Gradient	76196-700	Each
GeneTheatre Automated Liquid Handling System	76196-710	Each

Dilucup™ — A Most Elegant Solution in Serial Dilution Automation

HARDY DIAGNOSTICS
A Culture of Service™

Simple Format Puts Sophisticated Automation Technology Within Reach for Labs of Any Size

- The Dilushaker III and Dilucup™ Elegance serial dilution cups system ensures consistent results even when used by different lab technicians
- Practical and efficient — Dilucup Elegance serial dilution cups only require 3 physical touches versus seven for competing systems

Dilucup Elegance serial dilution cups are unique, prefilled containers with a sterile diluent used in combination with a purpose-built shaker: the Dilushaker III. Dilucup Elegance serial dilution cups allow laboratories to standardize and secure the serial dilution process in food microbiology.
Note: Power supply sold separately.

Description	Capacity	Cat. No.	Unit
Dilushaker III, 3 Rows, 7x3	Holds 21Cups	75860-388	Each
Dilushaker III, 6 Rows, 7x6	Holds 42 Cups	75860-392	Each
Dilushaker Power Supply, 12V	—	75860-400	Each
Dilucup Elegance Diluents			
Buffered Peptone Water	3 Rows; 32 Trays	75860-416	Each
Buffered Peptone Water	6 Rows; 16 Trays	75860-418	Each
Maximum Recovery Diluent	3 Rows; 32 Trays	75860-406	Each
Maximum Recovery Diluent	6 Rows; 16 Trays	75860-408	Each
Saline Solution	3 Rows; 32 Trays	75860-412	Each
Saline Solution	6 Rows; 16 Trays	75860-414	Each

CompactDry™

HARDY DIAGNOSTICS
A Culture of Service™

Simple and Effective System for Colony Counting

- **Easy-to-use —** Pour sample on plate, place cap on, and incubate.
- **Long Shelf Life —** 18 month shelf life from date of manufacture.
- **Easy-to-handle —** Rigid plastic frame with easy opening and removable lid.

CompactDry™ is a ready to use system reducing the time needed to perform microbial colony counting on food, beverage, meat, cosmetics, and raw materials. Simply place 1mL of sample onto the CompactDry™ plate and incubate. Colonies grow and develop specific colors due to chromogenic substrates, providing for easy organism differentiation and counting. Available for Salmonella, *E. Coli,* Listeria, Total Count, Yeast and Mold, and more.

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

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In 2017 Lucigen became the exclusive worldwide source, from manufacturing through distribution, of the Epicentre brand of products to ensure a consistent supply of these high quality, valuable and often unique products. See below for short list of some of the most popular products or find all the Lucigen/Epicentre products on **vwr.com** by just searching for "Epicentre." Then let the science begin.

Description	Size	Cat. No.	Unit
Ampligase® DNA Ligase Kit	1,000 Units	76081-604	Each
CircLigase™ ssDNA Ligase	1,000 Units	76081-606	Each
CircLigase II ssDNA Ligase	1,000 Units	76081-610	Each
FailSafe™ PCR PreMix Selection Kit (All 12 PreMixes)	60 Units (12 x 4 Rxn Each)	76081-632	Each
Hybridase™ Thermostable RNase H	500 Units	76081-726	Each
OmniCleave™ Endonuclease	50,000 Units	76081-706	Each
QuickExtract™ DNA Extraction Solution	50 mL	76081-766	Each
RNase R	250 Units	76081-690	Each

ÄKTA™ start

An Easy-to-Learn and Easy-to-Use System to Eliminate the Hassles of Manual Protein Purification

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GE Healthcare

- Purify tagged proteins and antibodies easily
- Gain insight from real-time monitoring
- Evaluate and share your results

From method creation and sample loading to fraction collection and data analysis, we've thought about how to make your work easier at every step. Let ÄKTA start transform your manual purification workflow into a convenient automated solution that delivers reproducible results.

Description	Cat. No.	Unit
ÄKTA start	10142-746	Each
UNICORN™ start 1.1 Control Software	75844-964	Each
Frac30 Fraction Collector	10142-748	Each

Antibody Purification

How to Combine Chromatography Techniques to Ensure the Right Balance Between Purity and Yield

Antibody purification protocols are typically challenged by three factors:

- Capturing as many antibodies as possible in the first step
- Controlling the degradation of the sample
- Removing the remaining impurities and minimizing the aggregate content
- Content is fixed, formatting is not.

Below are some recommendations for antibody purification that can be considered when planning your experiment. The choice of techniques and combinations will depend on the required purity and yields for your antibody of interest.

Before you start, you should carefully define your objectives and consider that in general, every added purification step will increase purity but decrease total protein recovery and yield.

What Do Antibody Purification Schemes Look Like?

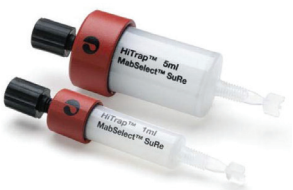
In the picture on the right, we describe a typical proven technique combination for the purification of antibodies.

- The 2-step protocol is the recommended best choice for research use.
- The 3-step protocol should be considered for scale-up or process development needs. SEC is not used as a final step to remove aggregates, fragments, or other impurities due to the limitations of sample volume. Instead, a combination of IEX steps is used.
- Steps in circles are optional and may be applied on an as needed basis.

Typical Columns for Purification of Antibodies

Affinity chromatography columns

- HiTrap™ Protein A HP
- HiTrap Protein G HP
- HiTrap MabSelect SuRe™



Ion Exchange Chromatography Columns

CIEX
- HiTrap Capto™ S ImpAct
- HiScreen™ Capto S ImpAct

AIEX:
- HiTrap Capto Q
- HiScreen Capto Q

Size Exclusion Chromatography Columns

- Superdex™ 200 Increase1,
- HiLoad™ Superdex 200 pg,
- HiPrep™ Sephacryl™ S-300

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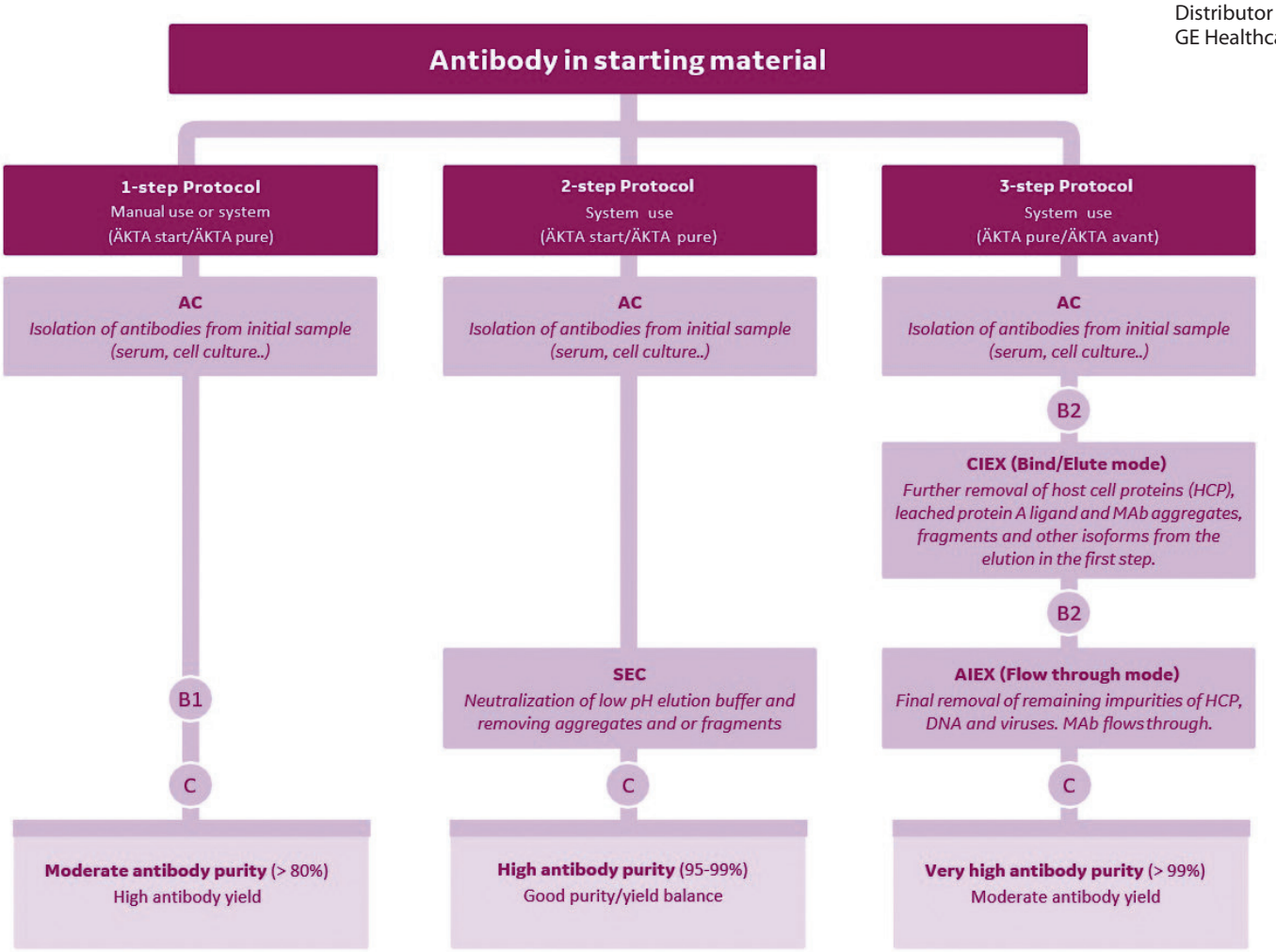

Fig.1. Combining techniques for antibody purification with regards to yield and purity.

C Concentration

B1 Buffer exchange to neutralize low pH elution buffer

B2 Buffer exchange to prepare for IEX

AC = affinity chromatography
AIEX = anion exchange chromatography
CIEX = cation ion exchange chromatography
SEC = size exclusion chromatography

Description	Cat. No.	Unit
HiTrap™ Protein A HP (1 x 1mL) and HiTrap Desalting (5 x 1mL)	10498-072	Each
HiTrap™ Protein G HP, 5mL	95056-034	Pack of 5
HiTrap MabSelect SuRe™, 1mL	95055-926	Pack of 5
HiTrap MabSelect SuRe, 5mL	95056-024	Each
HiTrap Capto™ S ImpAct, 1mL	10158-350	Pack of 5
HiScreen Capto S ImpAct, 5mL	10158-352	Pack of 5
HiTrap Capto Q, 1mL	97067-780	Pack of 5
HiScreen Capto Q, 5mL	97067-786	Each
Superdex™ 200 Increase 10/300GL	89497-272	Each

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