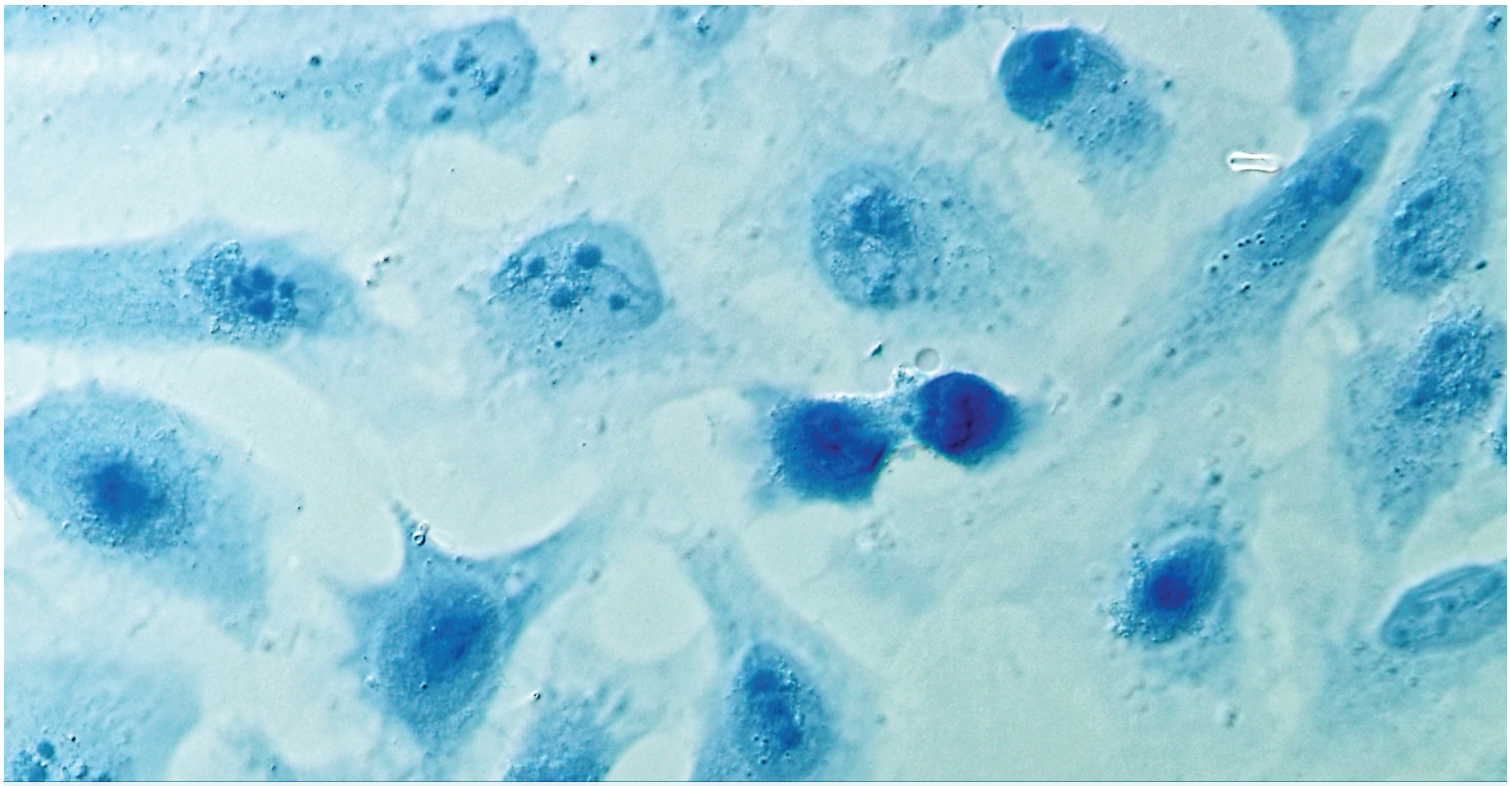
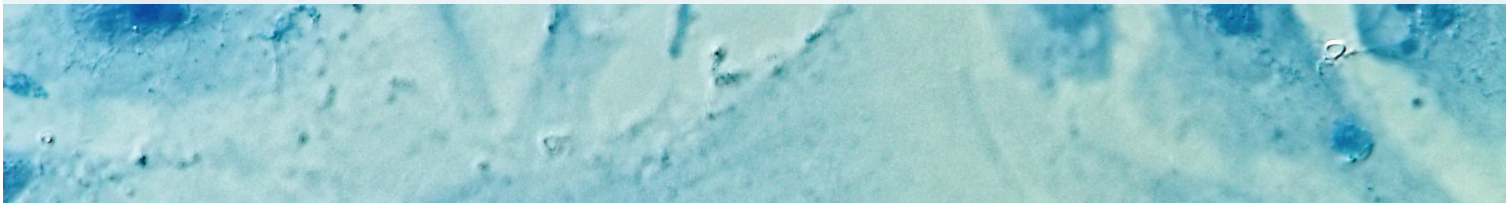


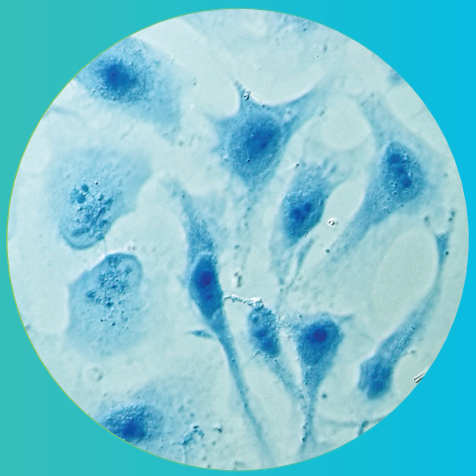


Focus: Cell Culture

Cell Culture for Analysis is the process of growing cells under controlled conditions in an artificial environment, providing excellent models for studying the normal physiology and biochemistry of the cells.

For more information on products that fit each step in this workflow, visit **vwr.com/cell-culture.**

2020



CORNING

Ramping up drug, vaccine and infectivity testing with airway organoids

By Elizabeth Abraham, Senior Product Manager and Hilary Sherman, Senior Applications Scientist for Corning Life Sciences

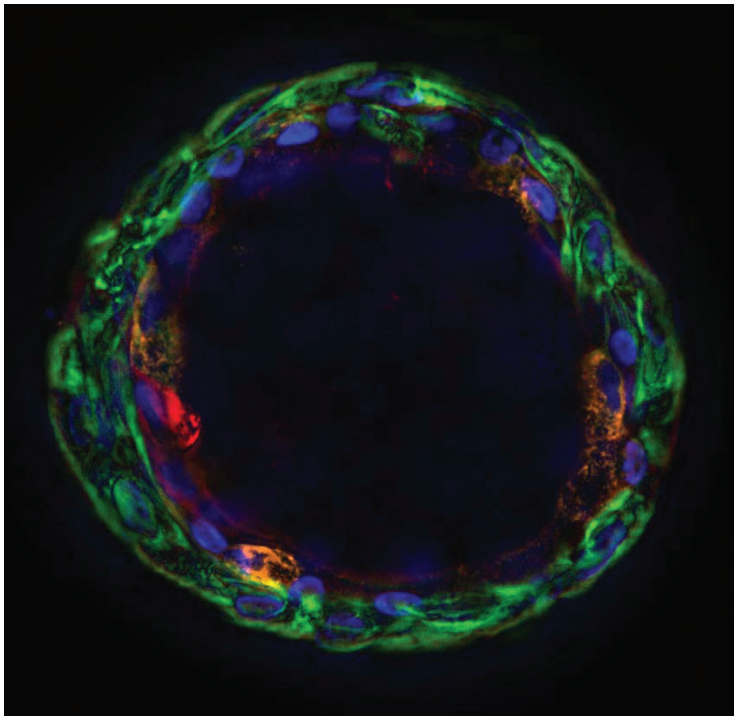
OVERCOMING THE LIMITATIONS OF 2D CELL CULTURE

Countless medical breakthroughs over the past century, including the discovery of new drugs, have begun with two-dimensional (2D) cell culture models. Most cell-based assays are still based on 2D culture models but the limitations of 2D monolayers cultured in flasks or plates is immediately obvious – cells, tissues, and organs exist in three dimensions, not two, and tissues of interest almost always consist of more than one cell type.

Three-dimensional (3D) cell culture has changed how biologists and medical researchers approach cell-based assays.[1] By providing greater physiologic relevance, 3D cultures narrow the divide between *in vitro* assays and live-animal[2] or human embryonic assays.[3] Organoids, in particular, show promise for their physiologic-like complexity and manufacturability. By enabling direct co-culture, or the self-directed differentiation of cultured precursor cells into distinct cell types, organoids capture many of the relevant interactions between test cells and their native niches, including chemical communication with neighboring structural cells and interactions with biological pathways that cells typically experience *in vivo*.[4]

OF ORGANOIDS AND SPHEROIDS

The terms "organoid" and "spheroid" are often used interchangeably but the differences with regard to physiologic relevance are germane to this discussion. Spheroids are typically free-floating cell aggregates generated from single, usually terminally differentiated cell types. Organoids arise from cultured induced pluripotent stem cells, organ-progenitor cells from adults, or cancer stem cells, all of which possess varying capacities for expansion and/or differentiation.[5] In addition, current organoid production protocols are compatible with homogeneous assays in which cells are grown and tested *in situ*.



Industrializing organoid production has been hampered by problems typically encountered with out-scaling living systems (think CHO culture for biomanufacturing), specifically throughput and consistency.[6] As we will learn, working with the right tools mitigates most of these issues.

SIGNIFICANCE OF AIRWAY ORGANOIDS

Organoids representing dozens of tissue types and subtypes are now available commercially, or accessible through published protocols. These include 3D models of liver, heart, pancreas, brain, GI tract, kidney,[7] and notably, as the world fights a coronavirus pandemic, of human airways.[8]

Lung and airway organoids are of interest for both drug and vaccine development and are valuable tools for studying infectivity in human respiratory diseases, particularly for challenging viral diseases like COVID-19. Airway organoids, which include multiple cell types, and which must account for cell–air, cell–fluid, and cell–cell interactions are every bit as complex as the physiologic entities they represent.[9]

Chinese and European researchers recently described a method for producing airway organoids for evaluating the infectivity of novel viruses in humans.[10] In addition to goblet, club, and basal epithelial cells, their technique produced organoids that included operational ciliated cells in numbers comparable to those observed in airways. In addition, the organoids secreted serine proteases which are required for influenza viruses to infect cells.

The investigators concluded that differentiated airway organoids "morphologically and functionally simulate human airway epithelium and as a proof of concept can discriminate human-infective influenza viruses from poorly human-infective viruses." Additionally, their method generates airway organoids that may be expanded "indefinitely" – a boon for industrialization – and display "remarkable phenotypic and genotypic stability."

HIGH-THROUGHPUT SCREENING

A recent presentation by scientists at Corning and NanoString Technologies described novel methods for high-throughput evaluation of gene expression in 3D airway organoids.[11] For the last 30 years, researchers have used permeable support materials to design air-liquid interface cultures to study airways. Through this method, airway progenitor cells differentiate into the main airway cell types, which eventually assemble into serviceable models for investigating asthma, cystic fibrosis, and other airway pathologies.

An earlier paper reported that airway organoids derived from primary human cells and grown in Matrigel also form 3D structures consisting of goblet, basal, and ciliated cells, but without the need for a permeable support structure.[12] This discovery freed investigators from the 96-well throughput limitations of the permeable support model. Multiplexing of up to 384 wells became feasible for the first time, with even higher throughputs possible.

Additionally, the new assay format permitted the use of a gene expression profile comparison tool, the nCounter® PlexSet™ assay, to compare gene expression in healthy and diseased tissue. PlexSet contributes further to throughput enhancements by requiring only cell lysis, thus eliminating several sample preparation steps associated with standard protocols.

Corning also supports other technology for situations where rapid-enough, low-throughput experimentation suffices. Transwell™ permeable supports in a 24-well format from Corning are cell culture systems consisting of permeable culture inserts in 24-well receiver plates or reservoirs.[13]

Both methods are useful in evaluating the infectivity of new respiratory viruses, as described by a Taiwanese group studying differentiated human airway organoids to assess infectivity of emerging influenza virus.[10] Infectivity, a critical characteristic of pandemic viruses, differs widely among pathogens that cross from animals to humans. To evaluate the ability of airway organoid models to predict this trait, investigators tested strains known to preferentially infect animal vectors or humans in both 2D and 3D cell culture models. As an improvement over conventional 2D methodology researchers generated the 2D airway models from 3D organoids that had previously differentiated into relevant airway component cells, particularly ciliated cells. Both models distinguished between viruses capable of infecting humans and those that were not.

News stories during the COVID-19 pandemic have reported on serious non-airway effects of the virus, even among recovered individuals.[14] Whether these conditions are due to the virus or were present before infection remains to be seen. That these conditions exist, however, suggests several interesting possibilities for further utilization of organoids.

We know that the COVID-19 virus affects the digestive tract, as 20% of those infected report at least one gastrointestinal symptom.[15] Citing previous work using airway organoids, a European research team confirmed in May, 2020, that SARS-CoV-2, the virus that causes COVID-19, infects human gut enterocytes.[16] Their infection model consisted of human gut progenitor cells differentiated under organoid-forming protocols favoring four distinct mature cell types normally found in human gut enterocytes. The human enterocyte organoid model further permitted the study of genomic alterations and expression of virus-related cytokines.

CONCLUSION

The near-exponential growth in research utilizing 3D cell cultures, and organoids, suggests that organoids are well-established in cell-based assays for drug discovery, toxicology, and basic biology.[8] Organoids provide physiologic relevance in a culture format appropriate for in situ assays. Due to the involvement of lungs in so many diseases – not limited to respiratory ailments – airway organoids represent a critical testing platform for the discovery of drugs to combat infection, cancer, and other lung diseases, to quantify infection and pathology, to monitor disease progression, and for vaccine discovery.

Organoid culture protocols based on Corning Life Sciences' extracellular matrices, reagents and cultureware provide a complete toolbox for exploring the capabilities of organoids in the study of airway diseases. Examples from the recent literature indicate that airway organoids may be produced in numbers sufficient for high-throughput screening, and relatively rapidly in response to imminent or existing threats from infectious diseases.

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Mycoplasma tested

MINIMUM ESSENTIAL MEDIUM (MOD.) (EMEM), CORNING®

BASED ON BASAL MEDIUM EAGLE (BME) FORMULATION TO AID CELL PROPAGATION

- Low endotoxin levels: < 0.25EU/mL
- Mycoplasma tested
- Liquid media products are formulated with cell culture grade WFI water
- Manufactured in a cGMP environment

It is a common cell culture medium developed from early work using Basal Medium Eagle (BME) with normal mammalian fibroblasts and certain subtypes of HeLa cells. When supplemented with serum, MEM has been used for cultivation of a wide variety of cells grown in monolayers, such as fibroblasts. This formulation contains Earle's salts and with or without L-glutamine.

Description	Cat. No.
Minimum Essential Medium (MEM), Sodium bicarbonate, NEAA, L-glutamine, sodium pyruvate	**89428-906**
Minimum Essential Medium (MEM) with Earle's salts and L-glutamine	**45000-382**
Minimum Essential Medium (MEM) with Earle's salts, without L-glutamine	**45000-386**
Minimum Essential Medium (MEM) with Earle's salts, without L-glutamine and phenol red	**45000-388**
Minimum Essential Medium (MEM), Powder with Earle's salts, without L-glutamine, phenol red, and sodium bicarbonate	**45000-586**
Minimum Essential Medium (MEM), Powder with Earle's salts and L-glutamine, without sodium bicarbonate	**45000-582**
Minimum Essential Medium (MEM), Powder (Autoclavable) with Earle's salts, without L-glutamine and sodium bicarbonate	**45000-592**
Minimum Essential Medium (MEM), Powder with Earle's salts, L-glutamine, and nonesstential amino acids, without sodium bicarbonate	**45000-594**

Time-saving technology using the power of the cloud

CELL COUNTER, CORNING

FIRST AUTOMATED CELL COUNTER TO UTILIZE ONLINE IMAGE PROCESSING WITH A CLOUD-BASED LEARNING ALGORITHM

- Provides fast and accurate results with no consumable costs
- Fits into the palm of a hand

The Corning Cell Counter can perform a single cell count in less than 3 seconds, which is much faster than any non-cloud based cell counting system. Utilizing a sophisticated Deep Neural Network for cell detection allows for optimal accuracy. When Trypan Blue is added, the system can also detect cell viability. This system utilizes a reusable counting chamber which is included, or a customer supplied hemocytometer.

The cell counter is easy to use. Simply connect to a Windows 10 computer or tablet and start the Cytosmart Cloud app. Place the loaded counting chamber on stage, focus on the cells, and press the Count button. The simplicity of the device allows anyone working in the lab to easily count cells without the need for extensive training.

Description	Cat. No.
Corning Cell Counter	**76200-994**

Ready-to-use

CANCER STEM CELL MEDIUM, PROMOCELL

DESIGNED FOR CULTURE OF SPHERE-FORMING CANCER CELL LINES AS TUMORSPHERES/MAMMOSPHERES

- Supports most commonly used cancer cell lines
- Allows extended serial tumorsphere passages (>10)
- High tumorsphere formation efficiency
- Serum-free and chemically defined
- Ready to use

The PromoCell Cancer Stem Cell Medium is a culture system designed for culture of sphere-forming cancer cell lines as tumorspheres/mammospheres. The formulation supports sustained cell proliferation, allowing for serial passage of the 3D culture.

The PromoCell Cancer Stem Cell Medium is ready-to-use and chemically defined providing a culture environment devoid of all stimuli originating from non-defined materials. It is particularly suitable as a cost-efficient tool for the standardized routine-culture of tumorspheres/mammospheres.

Description	Cat. No.
Cancer Stem Cell Medium, PromoCell	**75794-940**

Consistent performance at a great value

VWR® CELL CULTURE FLASKS

FLASKS ARE VACUUM GAS PLASMA TREATED FOR CONSISTENT CELL ATTACHMENT AND GROWTH

- Made of crystal clear virgin polystyrene
- Sterile and certified non-pyrogenic
- Volume graduations on both sides with special writing area
- Choice of vented or plug seal caps

Capacity	Culture Area	Packaging	Cat. No.
Standard Caps			
25 ml	12.5 cm^2	10/bag	**10062-870**
50 ml	25 cm^2	10/bag	**10062-874**
250 ml	75 cm^2	5/bag	**10062-862**
600 ml	182 cm^2	5/bag	**10062-866**
850 ml	300 cm^2	3/bag	**10062-886**
Vented Caps			
25 ml	12.5 cm^2	10/bag	**10062-868**
50 ml	25 cm^2	10/bag	**10062-872**
250 ml	75 cm^2	5/bag	**10062-860**
600 ml	182 cm^2	5/bag	**10062-864**
850 ml	300 cm^2	3/bag	**10062-884**

Cost-effective

AVANTOR® SERADIGM FB ESSENCE

COST-EFFECTIVE ALTERNATIVE TO FETAL BOVINE SERUM (FBS) AND PROVEN EFFECTIVE ACROSS A BROAD RANGE OF CELL TYPES AND ORIGINS

- 100% US Origin
- Cost effective alternative to FBS
- Effective across a broad range of cell types

FB Essence contains FBS, Bovine Calf Serum, Equine Serum, and a proprietary blend of supplements, vitamins, minerals, and growth factors.

Avantor Seradigm is a fully-integrated supplier of animal sera that provides the cell culture community with access to the most reliable supply of high performance, exceptional quality Fetal Bovine Serum (FBS) and cost-effective FBS alternatives. Our approach to sourcing and manufacturing is refreshingly unique and distinct from the competition, resulting in product performance and quality unlike any other.

Description	Size	Endotoxin	Hemoglobin	Cat. No.
FB Essence	50 mL	≤ 20 EU/mL	≤ 25 mg/dL	**10805-184**
FB Essence	500 mL	≤ 20 EU/mL	≤ 25 mg/dL	**10803-034**
FB Essence, Heat Inactivated	50 mL	≤ 20 EU/mL	≤ 25 mg/dL	**10799-384**
FB Essence, Heat Inactivated	500 mL	≤ 20 EU/mL	≤ 25 mg/dL	**10799-390**
FB Essence, Gamma Irradiated	500 mL	≤ 20 EU/mL	≤ 25 mg/dL	**10805-180**
FB Essence, Gamma Irradiated and Heat Inactivated	500 mL	≤ 20 EU/mL	≤ 25 mg/dL	**10805-182**

High quality, high performance

AVANTOR® SERADIGM PREMIUM GRADE FETAL BOVINE SERUM (FBS)

PREMIUM GRADE FBS IS WIDELY USED BY CELL CULTURISTS REQUIRING HIGH QUALITY, HIGH PERFORMANCE FBS FOR A WIDE VARIETY OF APPLICATIONS

- 100% US Origin
- High quality, high performance FBS for a variety of applications
- Preservation of native, growth-promoting factors and minimization of growth-inhibiting factors
- ISIA Certified Supply Chain for Traceability

Avantor Seradigm is a fully-integrated supplier of animal sera that provides the cell culture community with access to the most reliable supply of high performance, exceptional quality Fetal Bovine Serum (FBS) and cost-effective FBS alternatives.

Description	Size	Endotoxin	Hemoglobin	Cat. No.
Premium Grade Fetal Bovine Serum (FBS)	50 mL	≤ 5 EU/mL	≤ 25 mg/dL	**89510-194**
Premium Grade Fetal Bovine Serum (FBS)	500 mL	≤ 5 EU/mL	≤ 25 mg/dL	**97068-085**
Premium Grade Fetal Bovine Serum (FBS), Heat Inactivated	50 mL	≤ 5 EU/mL	≤ 25 mg/dL	**89510-196**
Premium Grade Fetal Bovine Serum (FBS), Heat Inactivated	500 mL	≤ 5 EU/mL	≤ 25 mg/dL	**97068-091**
Premium Grade Fetal Bovine Serum (FBS), Gamma Irradiated	500 mL	≤ 5 EU/mL	≤ 25 mg/dL	**97068-086**
Premium Grade Fetal Bovine Serum (FBS), Gamma Irradiated and Heat Inactivated	500 mL	≤ 5 EU/mL	≤ 25 mg/dL	**97068-088**

Convenience and security

VWR LIFE SCIENCE PENICILLIN:STREPTOMYCIN FOR TISSUE CULTURE, 100X

STERILE SOLUTION FOR TISSUE CULTURE APPLICATIONS

- 10,000 U/ml penicillin, 10 mg/ml streptomycin
- Sterile, endotoxin-tested, liquid-stable antibiotic solution
- Targets gram-positive and gram-negative bacteria

Inhibits cell wall synthesis and protein sythesis in gram-positive and gram-negative bacteria. Store in the freezer.

Size	Cat. No.
100 ml	97063-708

Surface-coating reagent

HUMAN VITRONECTIN MATRIX AND BUFFER KIT, PEPROTECH

PeproTech's Animal-Free Human Vitronectin Matrix and Buffer Kit is a cell culture surface-coating reagent for hESCs and hiPSCs in the undifferentiated, pluripotent state to be used in conjunction with PeproTech's Stem Cell Media.

- Animal-free
- Surface-coating reagent

Description	Size	Cat. No.
Human Vitronectin Matrix and Buffer Kit	1 Kit	75999-692

Complete media

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A XENO-FREE, HUMAN SERUM CONTAINING PHENOL RED-FREE COMPLETE MEDIA FORMULATION AND DESIGNED FOR THE *IN VITRO* EXPANSION OF ADIPOSE-DERIVED HUMAN MESENCHYMAL STEM CELLS (ADMSCS)

- Complete media
- Xeno-free
- Phenol red-free
- Contains human serum component
- Lyophilized growth factor component

US ONLY

Since its design, this media formulation has been shown to be suitable for the sustained growth of adipose tissue-derived, bone marrow-derived, umbilical cord-derived, placental-derived, and urine-derived MSCs in both adherent and suspension culture. For optimal results, culturing should be conducted on a surface coated with PeproTech's Animal-Free Human Vitronectin Matrix as a surface-coating reagent; however, other suitable extracellular matrix (ECM) proteins, such as fibronectin or vitronectin, can be used.

Description	Size	Cat. No.
PeproGrow™ hMSC Medium	500 mL	76305-716

This product is coming soon to Canada. Please contact your Avantor Life Science Specialist for information on availability or similar products available in your region.

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VWR® WATER JACKETED CO_2 INCUBATORS

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- 6.5 cu. ft. internal chamber volume
- Supplied with four removable stainless steel shelves and water pan
- Outstanding temperature stability and security with dual temperatures for over-temperature protection
- Fan-assisted airflow and in-chamber sensors maintain a uniform and stable growth environment
- Easy assembly and disassembly of interior without tools

Feel safe with a VWR CO_2 incubator that provides you with selection, performance and quality needed in today's cell culture laboratories. VWR's family of CO_2 incubators include water jacketed and air jacketed configurations to meet specific culturing preferences and a wide range of solutions to meet capacity and budget needs.

Description	Cat. No.
Water Jacketed CO2 Incubator, TC Sensor	10810-744
Water Jacketed CO2 Incubator, IR Sensor	10810-878
Water Jacketed CO2 Incubator, TC Sensor, Dual	10810-884
Water Jacketed CO2 Incubator, IR Sensor, Dual	10810-886

Magnetic 3D cell culture

CELLSTAR® CELL-REPELLENT SURFACE MICROPLATES AND DISHES, GREINER BIO-ONE

INTRODUCING A NEW SURFACE CHEMISTRY DESIGNED TO EFFECTIVELY INHIBIT CELL ADHESION

- Nonpyrogenic
- Free of RNase, DNase, and human DNA
- Cell-repellent surface
- Ideal substrate for native cell culture experiments

Description	Size	Bottom Style	Color	Sterility	Cat. No.
Multiwell Microplate					
Cell-Repellent 6-Well Microplate with Lid		Flat-Bottom	Clear	Sterile	30618-022
Cell-Repellent 24-Well Microplate with Lid		Flat-bottom/Chimney Well	Clear	Sterile	10014-320
Cell-Repellent 48-Well Microplate with Lid		Flat-bottom	Clear	Sterile	10014-322
Cell-Repellent 96-Well Microplate with Lid		Flat-bottom/Chimney Well	Black, µClear®	Sterile	10859-852
Cell-Repellent 384-Well Microplate with Lid		Flat-bottom	Clear	Sterile	10014-324
Cell-Repellent 384-Well Microplate with Lid		Flat-bottom	Black, µClear®	Sterile	10014-326
Cell Culture Dishes					
Cell-Repellent Cell Culture Dish with Lid	35×10 mm		Clear	Sterile	10014-312
Cell-Repellent Cell Culture Dish with Lid	60×15 mm		Clear	Sterile	10014-314

Culturing suspension HEK 293 cells for the manufacture of recombinant viral vectors

Tips for maintaining suspension HEK 293 cells when producing virus via transfection

By Austin Storck, Jim Ludtke, and Laura Juckem, Mirus Bio LLC, Madison, Wisconsin USA

INTRODUCTION

Suspension HEK 293 cells are commonly used for protein and virus production because they are well characterized, grow heartily in a variety of cell culture media (e.g. serum-free conditions) and are amenable to scale-up in biomanufacturing processes. These workhorses of the cell culture world are often unnoticed, but they still require proper care for optimal performance in your next experiment, whether it be for basic science research or for production of biotherapeutics. Here, we describe key cell culture techniques to promote high titer recombinant lentivirus and adeno-associated virus (AAV) production in suspension HEK 293 cells when generated using transient transfection.

ROUTINE CELL SPLITTING

When culturing suspension HEK 293 cells, we recommend performing cell counts and evaluating viability daily to ensure cells are exhibiting a healthy cell profile. Healthy cells double every 24 hours and are ≥ 95% viable by trypan blue exclusion. Cells should be split routinely to a lower cell density (e.g. 5×10^5 cells/ml) to ensure that the cells do not overgrow. Established suspension HEK 293 cultures grow best when the cell counts are maintained between 5×10^5 and 4×10^6 cells/ml. Employing a consistent 3-day split schedule (i.e. Monday, Wednesday, Friday) to keep them within this range of cell densities will allow for optimal cellular growth and performance (Figure 1). Ensure that the cells are split to 5×10^5 cell/ml on Friday to prevent the cells from overgrowing during the weekend.

	Mon	Tues	Wed	Thur	Fri	Sat	Sun
Routine Target Cell Density (cells/ml)	$5x10^5$	no split	$5x10^5$	no split	$5x10^5$	no split	no split
Are cells ready for transfection?	x	✓	✓	✓	✓	x	x
	On the day before transfection, prepare cells by resuspending to a target cell density of $2x10^6$ cells/ml					no split	no split
	Healthy suspension HEK 293 cell cultures have >95% viability and double every 24 hours. On the day of transfection with *Trans*IT-VirusGEN® Transfection Reagent, split cells to $4x10^6$ cells/ml						

FIGURE 1: **Example HEK 293 Cell Splitting Schedule.** Employing a consistent 3-day split schedule will ensure suspension HEK 293 cells are growing optimally. The day before you plan to transfect cells for virus production with *Trans*IT-VirusGEN® Transfection Reagent, resuspend the cells in fresh medium at a density of 2×10^6 cells/ml. See Preparation for High Density Transfection on page 12.

There may be times when higher cell densities are necessary, such as for some transfection protocols. Allowing your cultures to reach densities of up to 6×10^6 cells/ml for transfection should not adversely affect the cells if they are provided with sufficient nutrients and if growth in high density is only experienced occasionally.

PREPARATION FOR HIGH DENSITY TRANSFECTION

In some circumstances — such as in high titer lentivirus production — you may need to transfect your suspension HEK 293 cells at a high cell density (e.g. 4×10^6 cells/ml) to achieve maximal viral titers. To ensure your cells are growing and healthy at the high density that is required, prepare the cells the day before transfection as follows:

01. Approximately 24 hours before transfection, pellet suspension HEK 293 cells by centrifugation at 300xg for 5 minutes.
02. Resuspend the cells in fresh medium at a density of 2×10^6 cells/ml.

This gentle pelleting of cells and media change will guarantee that your HEK 293 cells achieve the necessary growth for high density transfection the following day.

SERUM-FREE COMPLETE GROWTH MEDIA

A variety of serum-free complete growth media are commercially available for suspension HEK 293 cells. Each medium has unique attributes concerning its ability to support high or low cell density growth and transfection compatibility. Some serum-free media contain polyanions such as dextran sulfate or heparin, which can inhibit transfection. Culturing cells in polyanion-containing media is not recommended if transfection is to be performed. If use of polyanion-containing media is necessary in your workflow, perform the transfection in a medium without polyanions and replace it 4 to 24 hours post-transfection.

For most applications, a media change should not affect transfection efficiency if performed at least 4 hours post-transfection. *Trans*IT-VirusGEN® Transfection Reagent is compatible with multiple serum-free media and does *not* require a medium change at any time during or after transfection for optimal transfection efficiency.

ADAPTION OF SUSPENSION HEK 293 CELLS TO DIFFERENT SERUM-FREE GROWTH MEDIA

When adapting cells to a new serum-free growth medium a gradual, stepwise approach is recommended. Sub-culture the suspension cells in an increasing ratio of the new target medium relative to the current medium. To start we recommend using 75% volume of the current serum-free media formulation and 25% target media formulation (v/v). If cells are not doubling as expected, and/or viability is < 95%, do not increase the percentage of the target medium in the culture. Cell doubling time should be normal and viability over 95% before each stepwise increase. After successful adaptation in 100% target medium, cells may be cryogenically stored in the target medium; henceforth, they can be cultured in the target medium immediately out of thaw.

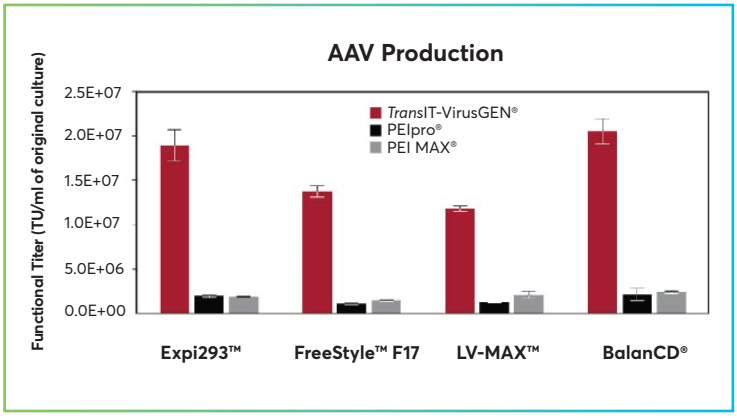


FIGURE 2: TransIT-VirusGEN® Transfection Reagent Is Compatible with Multiple Serum-Free Complete Growth Media for AAV Production. Suspension Expi293F™ cells (Thermo Fisher) adapted to the indicated growth media formulations were transfected for AAV production with pAAV-hrGFP, pAAV-RC, and pAAV-Helper (1:1:1 ratio, 1.5 µg/ml, Agilent Technologies) using TransIT-VirusGEN® (2:1, vol:wt), PEIpro® (1:1, Polyplus), or PEI MAX® (2:1, Polysciences). Harvested AAV was used to transduce HT-1080 and GFP expression was measured using guava easyCyte™ 5HT Flow Cytometer. Functional titers were measured from virus dilutions with less than 20% GFP positive cells. The error bars represent the range of duplicate wells.

This approach was utilized for Expi293F™ cells grown in Expi293 Expression Medium (Thermo Fisher) for three other different commercially available serum-free growth media formulations: FreeStyle™ F17 Expression Medium (Thermo Fisher), LV-MAX Production Medium (Thermo Fisher) or BalanCD HEK293 Medium (Irvine Scientific). The adapted cultures were then transfected with *Trans*IT-VirusGEN® Transfection Reagent (Mirus Bio), PEIpro® (Polyplus Transfection) or PEI MAX® (Polysciences) to produce recombinant AAV2 encoding green fluorescent protein (GFP). Functional virus titers were measured following the transduction of HT-1080 (Figure 2). High levels of recombinant AAV2 were produced when the cells adapted to all four media formulations were transfected with the *Trans*IT-VirusGEN® Transfection Reagent.

ADDRESSING CELL AGGREGATION

Aggregates of cells or "clumps" may form during normal cell culture depending on the growth medium and attributes of the specific HEK 293 cell line. Clumps will not harm your culture but can aggregate into larger particles that are undesirable for downstream applications such as transfection, filtration or protein purification. Cell clumping also creates difficulties for accurate cell counting and uptake of nutrients and/or transfection complexes, which may not be readily available to the cells in the interior of the cell mass. Polyanions, such as dextran sulfate or heparin, can help decrease clumping, but can also inhibit transfection.

Clumping of suspension HEK 293 cultures can frequently be mitigated by adding Poloxamer 188 (0.1-0.2% v/v final concentration), and it will not interfere with transfection. If cell clumping persists, the following gentle decanting protocol can help to reduce the number of clumps in your culture:

01. During normal cell maintenance, allow your suspension HEK 293 cells to settle for 5 minutes without agitation. Large cell clumps should sink to the bottom of the flask.
02. Gently transfer the top half of the cell suspension into a new flask with a sterile pipette. Discard the cell aggregates that remain in the old flask.
03. Add fresh media to reach your desired cell density.

This procedure may be repeated several times to decrease the cell clumps in a culture.

SUMMARY

Healthy HEK 293 cell cultures generate higher yields of recombinant lentivirus and adeno-associated virus when using a transient transfection method. Different serum-free growth media formulations can be utilized as part of your manufacturing workflow as long as the cells are gradually adapted to the new media formulation. Thoughtful maintenance and use of the culture techniques outlined here will lead to increased performance from your suspension HEK 293 cells.

Reliable, scalable, and flexible

TRANSIT®-VIRUSGEN™ TRANSFECTION REAGENT, MIRUS BIO

DESIGNED TO ENHANCE DELIVERY OF PACKAGING AND TRANSFER VECTORS TO ADHERENT AND SUSPENSION HEK 293 CELL TYPES TO INCREASE RECOMBINANT ADENO-ASSOCIATED VIRUS (AAV) AND LENTIVIRUS PRODUCTION

US ONLY

- **Reliable –** Consistent high virus titer production
- **Scalable –** Efficient across different formats
- **Flexible –** Address different virus and cell culture systems

Description	Size	Cat. No.
TransIT-VirusGEN Transfection Reagent		
TransIT®-VirusGEN™, DNA transfection reagent for lentivirus and AAV production	0.75 ml	**76183-204**
TransIT®-VirusGEN™, DNA transfection reagent for lentivirus and AAV production	5 x 1.5 ml	**76183-206**
TransIT®-VirusGEN™, DNA transfection reagent for lentivirus and AAV production	10 x 1.5 ml	**76183-208**
TransIT®-VirusGEN™, DNA transfection reagent for lentivirus and AAV production	0.3 ml	**76183-246**
TransIT®-VirusGEN™, DNA transfection reagent for lentivirus and AAV production	1.5 ml	**76193-582**
TransIT-VirusGEN SELECT Transfection Reagent		
TransIT-VirusGEN SELECT, tested to support preclinical and early phase clinical trials	30 ml	**76333-948**

These products are not available in Canada. Please contact your Avantor Life Science Specialist to learn more about similar offerings available in your region.

The camera and fluorescence upgradeable inverted microscope

LEICA DMIL INVERTED MICROSCOPE PACKAGES, LEICA MICROSYSTEMS

FLUORESCENCE-READY DIGITAL IMAGING SOLUTION DESIGNED FOR USE IN A VARIETY OF LABORATORY SETTINGS

- Inverted laboratory microscope with LED illumination
- High-performance optics
- Ergonomic design
- 3W white light LED illumination

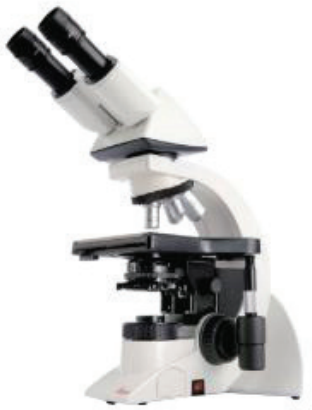
The DM IL LED Inverted Microscope Lab Package is a robust inverted microscope with LED illumination designed for years of use without the need to replace bulbs.

The DM IL LED is fully configured for both brightfield and fluorescence imaging. It features an LED light source comprised of 5 solid state sources operating simultaneously to produce up to 3W of White Light. The DM IL features long working objectives from 4x to 40x. The 10x, 20x and 40x each have phase rings inscribed on them that are matched to the Phase-1, Phase-2 and phase-3 Light Rings contained within a single slider. The DM IL is available either with a camera port or stand alone. When properly configured, the DM IL can be combined with a selection of cameras from the Leica camera portfolio. These include the 5MP Leica MC170, the 3MP Leica DMC2900 and the 1.3MP Leica DFC3000 G. Additional stage accessories are available to fit most imaging applications.

Description	Cat. No.
Microscope Stand	
Leica DMiL LED Base Package, Fluorescence and Camera Upgradeable	**76382-982**
Leica DMiL LED Base Package, Fluorescence Upgradeable	**76382-980**
Camera Mount	
C-Mount HC 0.55×	**10037-632**

Description	Cat. No.
Accessories	
MC170 HD camera kit, color	**10037-578**
DFC3000 G monochrome camera kit	**76382-984**
DMC2900 camera kit, color	**76382-986**

Visit **vwr.com** to learn more or to find additional accessories.



High efficiency electroporation

INGENIO® EZPORATOR® ELECTROPORATION SYSTEM, MIRUS BIO

US ONLY

DESIGNED TO BE USED WITH THE INGENIO® ELECTROPORATION SOLUTION FOR MANY HARD-TO-TRANSFECT CELL TYPES

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

Description	Cat. No.
Ingenio® EZporator® Electroporation System	**76304-532**

These products are not available in Canada. Please contact your Avantor Life Science Specialist to learn more about similar offerings available in your region.

More clones in less time

CLONEPIX™ 2 MAMMALIAN COLONY PICKER, MOLECULAR DEVICES

REDEFINE CLONE SCREENING AND SELECTION: TRANSFORM YOUR CELL LINE DEVELOPMENT WORKFLOW

- Screen more clones in less time
- Accurate, automatic colony picking avoids errors associated with limiting dilution
- Excellent image quality allows for screening of stable, high-producing clones
- Increased productivity of a cell line development workflow
- Add FL and white light imaging
- > 200 clones per hour

The ClonePix™ 2 Mammalian Colony Picker is a fully automated system for the selection of high-value clones used in antibody discovery and cell line development. Hybridoma, CHO cells, and other cell types are imaged and selected based on user-defined parameters. Plate handling, barcode reading, and picking are all fully integrated. All data, including images, are saved for downstream analysis.

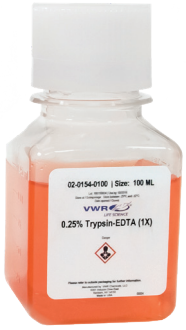
Description_NA	Cat. No.
ClonePix™ 2 mammalian colony picker	**76404-534**

Aids in harvesting and passaging

VWR LIFE SCIENCE TRYPSIN (0.25%) EDTA (1X)

- Used to release adherent cells
- Aids in harvesting and passaging

Contains 0.001% phenol red, 0.25% Trypsin, 0.04% EDTA.



Description	Volume	Cat. No.
Trypsin (0.25%) EDTA (1X)	100 mL	**VWRL0154-0100**

Check your cell viability

VWR LIFE SCIENCE TRYPAN BLUE, 0.4% IN AQUEOUS SOLUTION, READY-TO-USE, STERILE

A pre-mixed dye solution used in cell culture applications to determine cell viability. A researcher can remove a sample of cells from culture and combine in a 1:1 ratio with the Trypan Blue solution. Under the microscope, dead cells will appear a blue color and viable cells will appear clear and translucent. Using a hemacytometer (gridded microscope slide), a researcher can quantify the percentage of dead cells within a population. Stained cells are ready for counting within five minutes.

Size	Cat. No.
100 ml	**97063-702**

Cell-freezing media

DIMETHYL SULPHOXIDE ≥99.9%, ULTRA PURE GRADE

CAS 67-68-5
$(CH_3)_2SO$
Boiling Pt: 189 °C (1013 hPa)
Flash Pt: 87 °C

M.W. 78,14 g/mol
Melting Pt: 18,5 °C
Density: 1,101 g/cm^3 (20 °C)

Size	Cat. No.
5x10 ml	**97063-136**

Eliminate variability

VWR LIFE SCIENCE SERAFREE™ CRYOPRESERVATION MEDIA

- Sterile and endotoxin tested
- Reduces potential for transmission of infectious agents
- Eliminates regulatory compliance requirements associated with use of animal products
- Cost effective

SeraFree™ Cryopreservation Media is a ready-to-use freezing media for cryopreservation of adherent or suspension cultured cells. The animal-free RPMI or DMEM based media composition eliminates batch-to-batch variability and are optimized for cell viability and cell growth after thawing.

Description	Size	Cat. No.
SeraFree™ DMEM Cryopreservation Media	50 mL	**97063-278**
SeraFree™ Cryopreservation Media (RPMI)	50 mL	**97064-276**

Convenient and customizable

VWR® CRYOPRO® LIQUID DEWARS, L SERIES

DESIGNED FOR EFFICIENT AND SAFE HANDLING OF LIQUID NITROGEN

- Convenient storage and dispensation of liquid nitrogen
- Accessories allow for customization of dewars depending on application

Large or small quantities of liquid nitrogen can be conveniently stored and dispensed with these dewars and accessories. Accessories for pouring and transferring liquid nitrogen allow you to customize the dewars to your application.

Description	Capacityw	O.D.×H	Neck Diameter	Static Evaporation Rate	Weight Empty	Weight Full	Cat. No.
L-4 Liquid Dewar	3.8 L (1 gal.)	18.5 x 42.6 cm (7 1/4 x 16 3/4")	3.5 cm (1 3/8")	0.19 L/Day	2.7 kg (6 lbs.)	6 kg (13 lbs.)	**82021-112**
L-5 Liquid Dewar	5 L (1.3 gal.)	22.2 x 46.2 cm (8 3/4 x 18 3/16")	5.6 cm (2 3/16")	0.15 L/Day	4 kg (8.8 lbs.)	8 kg (17.6 lbs.)	**55709-234**
L-10 Liquid Dewar	10 L (2.6 gal.)	26 x 54.6 cm (10 1/4 x 21 1/2")	5.6 cm (2 3/16")	0.18 L/Day	6 kg (13.2 lbs.)	14 kg (30.9 lbs.)	**55709-236**
L-20 Liquid Dewar	21 L (5.5 gal.)	36.8 x 62.7 cm (14 1/2 x 24 11/16")	5.1 cm (2")	0.18 L/Day	9 kg (19.8 lbs.)	26 kg (57.3 lbs.)	**55709-238**
L-30 Liquid Dewar	32 L (8.45 gal.)	43.2 x 61.1 cm (17 x 24")	6.4 cm (2 1/2")	0.22 L/Day	12 kg (26.4 lbs.)	38 kg (83.8 lbs.)	**55709-240**
L-50 Liquid Dewar	50 L (13.2 gal.)	43.2 x 77.9 cm (17 x 30 11/16")	6.4 cm (2 1/2")	0.49 L/Day	15 kg (33 lbs.)	56 kg (123.5 lbs.)	**55709-242**

For accessories, please visit **vwr.com**.

Choices to keep your cells safe

VWR® CRYOGENIC VIALS, INTERNALLY OR EXTERNALLY THREADED

DESIGNED FOR STORING BIOLOGICAL MATERIAL CELLS AT TEMPERATURES AS LOW AS -196°C

- Internal or external threaded closure options available
- White marking area and printed graduations on all vials
- Sterilized by gamma radiation

Closures and tubes are made of polypropylene with the same coefficient of expansion, which further enhances the leakproof qualities of these vials at various temperatures.

Description	Capacity	O.D.×H	Sterility_NA	Cat. No.
Internally Threaded with Washer				
Self-Standing Vials with Silicone Washer Seal Cap	1.2 mL	12.5 x 41 mm	Sterile	**10018-738**
Self-Standing Vials with Silicone Washer Seal Cap	2 mL	12.5 x 49 mm	Sterile	**10018-760**
Self-Standing Vials with Silicone Washer Seal Cap	4 mL	12.5 x 72 mm	Sterile	**10018-824**
Self-Standing Vials with Silicone Washer Seal Cap	5 mL	12.5 x 91 mm	Sterile	**10018-780**
Internally Threaded with Red O-Ring				
Self-Standing Vials with Silicone O-Ring Seal Cap	1.2 mL	12.5 x 41 mm	Sterile	**10018-736**
Self-Standing Vials with Silicone O-Ring Seal Cap	2 mL	12.5 x 49 mm	Sterile	**10018-758**
Self-Standing Vials with Silicone O-Ring Seal Cap	4 mL	12.5 x 72 mm	Sterile	**10018-822**
Self-Standing Vials with Silicone O-Ring Seal Cap	5 mL	12.5 x 90 mm	Sterile	**10018-778**

For additional sizes or thread options, visit **vwr.com**.

Controlled-rate freezing

COOLCELL® CONTAINERS, CORNING®

PROVIDES ALCOHOL-FREE FREEZING IN A -80°C FREEZER AT THE RATE OF -1°C/MINUTE; IDEAL FOR CRYOPRESERVATION OF MOST CELLS AND CELL LINES

- For 12 or 30 standard cryogenic vials
- Radially symmetric for uniform vial freezing
- Numbered wells for easy sample identification
- Beveled lid for secure gripping and easy opening
- To remove vials tip the device upside down

Size	Capacity	Color	Cat. No.
11.7 x 10.9 cm	Twelve 1 to 2 mL Cryogenic Vials	Purple	**75779-712**
9.5 x 14.5 cm	Twelve 3 to 5 mL Cryogenic Vials	Purple	**75779-722**
16.5 x 11.5 cm	Thirty 1 or 2 mL Cryogenic Vials	Green	**75779-816**
13.9 x 10.5 cm	Twelve 2 mL Serum Vials	Purple	**75779-820**
12.1 x 9.8 cm	Six 10 mL Serum Vials	Purple	**75779-822**
11.7 x 10.9 cm	Twelve 1 to 2 mL Cryogenic Vials	Purple, Green, Orange, Pink	**76180-494**

Cryopreservation of human and animal primary cells

CRYO-SFM FREEZING MEDIUM, PROMOCELL

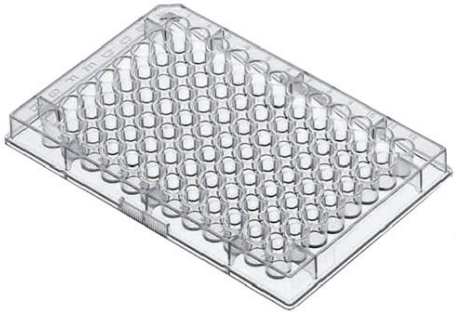
AN ANIMAL COMPONENT-FREE, CHEMICALLY DEFINED MEDIUM DEVELOPED FOR THE CRYOPRESERVATION OF HUMAN AND ANIMAL PRIMARY CELLS

Note: Each freeze/thaw cycle leads to a loss in proliferation potential and cell number, particularly when working with fragile primary cells. If freezing the cells, please do so at as low a passage number as possible.

Recommended for human primary cells, animal primary cells, established cell lines, induced pluripotent stem cells, embryonic stem cells

Description	Size	Cat. No.
Cryo-SFM	30 mL	**10172-412**
Cryo-SFM	125 mL	**10172-414**

Consistent cell attachment, spreading, and growth



VWR® CELL CULTURE PLATES, STERILE

TREATED FOR INCREASED CELL ATTACHMENT

- Available with 6 different growth surface areas
- Uniform well volume ensures an equal growth surface area
- Flat well bottom and round bottom plates available
- Well surface is smooth and free from striation to maximize usable growth area
- Raised rims on wells with uniform rings on the lid to reduce evaporation
- Single position lid reduces the risk of cross-contamination and handling mistakes
- Wells are labeled with alphanumeric code for easy identification
- Suitable for all common instruments and automation
- Sterilized by electron beam irradiation
- DNase- and RNase-free, and non-pyrogenic

No. of Wells	Bottom Style	Growth Area	Cat. No.
6-Well	Flat Bottom	1.90-2.90 cm^2	**10861-696**
12-Well	Flat Bottom	0.76-1.14 cm^2	**10861-698**
24-Well	Flat Bottom	0.38-0.57 cm^2	**10861-700**
48-Well	Flat Bottom	0.19-0.29 cm^2	**10861-702**
96-Well	Flat Bottom	0.075-0.2 cm^2	**10861-666**
96-Well	U Bottom	0.1-0.2 cm^2	**10861-668**

Transfection selector

VISIT VWR.COM/ TRANSFECTION SELECTION

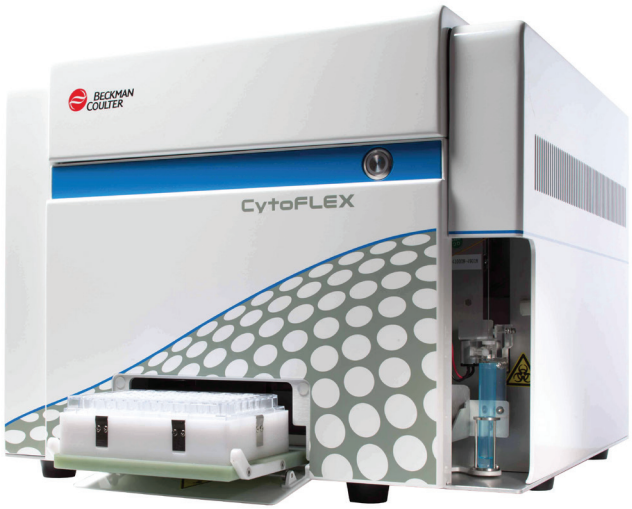
Search hundreds of transfection reagents for bioproduction, *in vivo* work, viral production, DNA and siRNA transfection, CRISPR/Cas 9, and more!

SEARCH for specialized transfection reagents

ADDRESS rare cell cultures, neurons, and primary cells

FIND additional products such as electroporation and reagents

Powerful analysis where and when you need it



CYTOFLEX FLOW CYTOMETER, BECKMAN COULTER

DESIGNED TO DELIVER SUPERIOR PERFORMANCE WITH EASE OF INSTALLATION AND OPERATION FOR RESEARCH APPLICATIONS

Simplified system setup, data acquisition, analysis, and export of experimental results are integrated into a complete workflow solution with CytExpert software.

The CytoFLEX includes 13 band pass filters which can be repositioned as needed, and it is available with different configurations to provide the ultimate in application flexibility, including optional 96-well Plate Loader. Activate the number of channels needed initially and add channels later as research needs grow.

US ONLY

Dimensions	16.7 W x 13.4 D x 14" H
Operating System Compatibility	Windows® 7 Professional 64-bit
Operating Temperature	59 – 80.6 °F, non-condensing
Power	150 – 250W
Sensitivity	FITC <30 MESF; PE <10 MESF
Voltage	100 – 240V
Weight	51.6 lbs. (without Plate Loader), 61.7 lbs. (with Plate Loader)

Description	Cat. No.
CytoFLEX System B4-RO-VO	**76330-530**
CytoFLEX System B3-R1-VO	**76330-090**
CytoFLEX System B2-RO-V2	**76330-092**
CytoFLEX System B2-R2-VO	**76330-094**

Description	Cat. No.
Accessories	
CytoFLEX* Sheath Fluid	**76183-428**
Sheath Filter	**76183-334**
CytoFLEX Sheath Sensor	**76183-340**
Sheath Bottle Only	**76183-436**

These specific models are coming soon to Canada. Please contact your Avantor Life Science Specialist to learn about availability or similar models available in your region.

28195-KK-JS

1120 10M Lit. No. 931220