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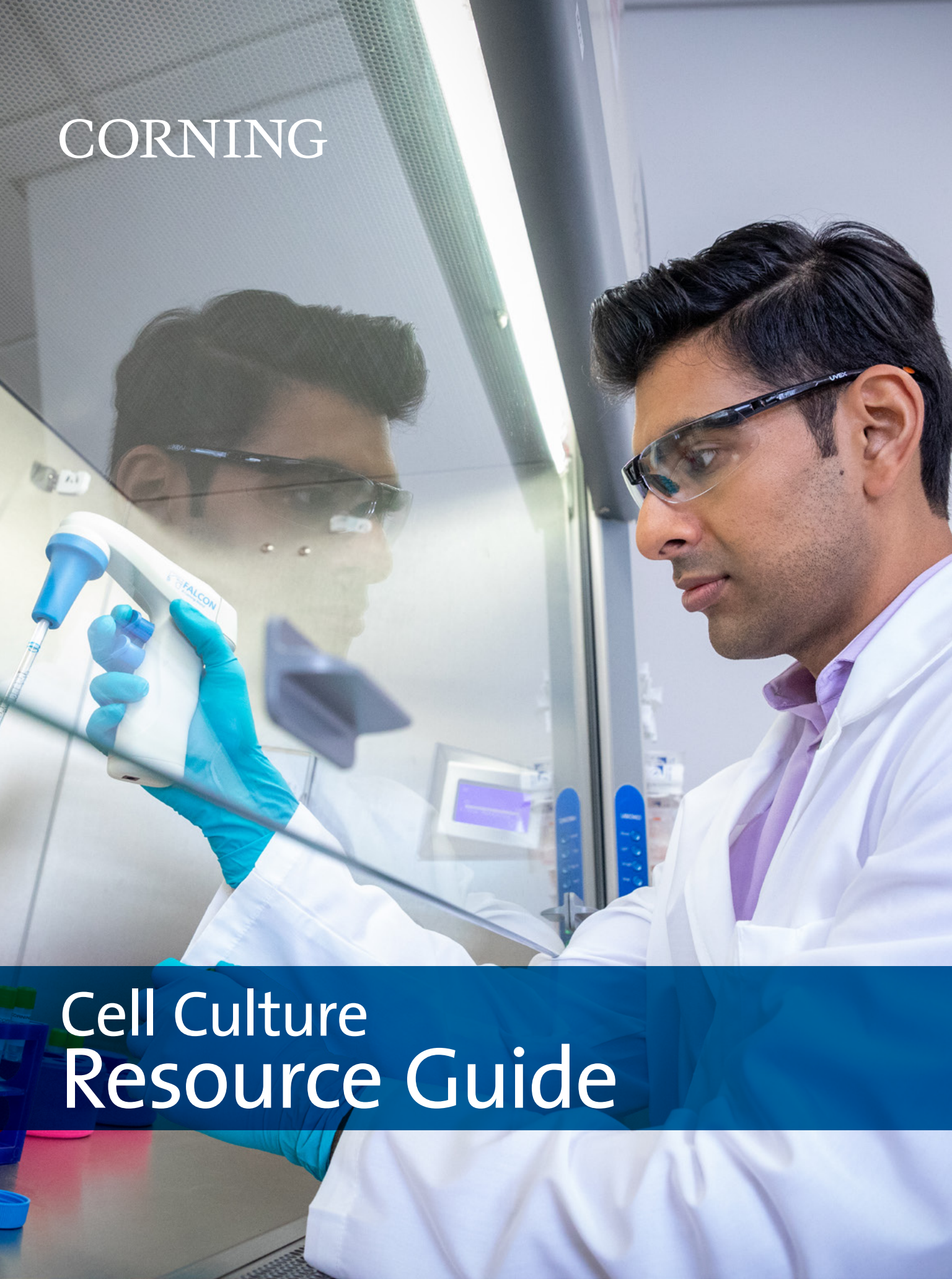

Cell Culture Resource Guide

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Introduction

Cell culturing is a foundational tool within the life sciences, playing a critical role in answering basic scientific and translation research questions across disciplines. Maintaining healthy cultures is vital to obtaining reliable, high-quality data. This eBook explores considerations and troubleshooting tips from cell stock cryopreservation through confluency.

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Guide to Adherent Cell Culture Basics: Seeding, Expanding, and Harvesting

Cell culturing is hard work. Because it's a highly technical process, there's a lot that can go wrong when you take cells from one source and manipulate them in another, from contamination to lackluster growth.

But there's also a lot that can go right. Over the past 100-plus years, adherent cell culture experiments have contributed to some of the most revolutionary advances in modern medicine, advancing our understanding of diseases like cancer and supporting the development of safe and effective therapeutics.

Fortunately, there's a lot more known about culturing now than when the *in vitro* pioneer, Ross Harrison, analyzed frog tissue on a glass slide in the early 1900s. In this guide, we share the basics learned from more than a century of tinkering with living cells, including how to seed, expand, and harvest cell cultures.

Cell seeding from a cryogenic state

In buying cell culture lines, products come cryopreserved, which is necessary to maintain cell viability long-term. From that state, you'll need to thaw out and seed the cells, which shouldn't be rushed, even though it happens fast. And yet, this important step often *does* get hurried and sometimes overlooked, which risks contamination and doesn't give the cells the good, sterile start they need.

Here is the correct way to conduct the cell seeding process:

- Get all of your supplies ready. You'll need your cryopreserved vial, a beaker or water bath filled with pre-warmed water, and a flask with pre-equilibrated media. You'll also need a conical vial filled with media

if you intend to spin down the cells and remove the DSMO or other cryoprotectant.

- Warm the vial. Gently swirl the cryopreserved vial in the beaker of pre-warmed water until the contents are almost completely thawed. Then, clean the vial with an alcohol wipe to reduce contamination risk.

- Seed your cells. Using a pipette, remove the cells from the cryovial and transfer them to the flask. Then, pipette the flask contents up and down to mix the solution for easy seeding. With that, your cells are thawed, transferred, and ready for the incubator (or the centrifuge for DSMO removal).

Scaling up with cellular expansion

When it's time to scale up your cells, you'll need the right cell expansion approach. Cellular expansion is a necessary part of cell culture that relies on both efficiency and consistency. If you follow an inefficient workflow, you'll likely drive up the costs of supplies and labor. If you follow an inconsistent workflow, you could overstress your cells and kill them.

Striking the right balance will depend on your vessel choice. Multilayer options, such as the Corning® HYPERFlask® vessel, can help life scientists establish friendly conditions for faster growth in a smaller space, and also with less labor and contamination risk.

Because not all vessels have the same growth area, researchers will typically think in terms of cells/cm^2 yield when they are determining their vessel choice and the amount of associated reagents. They will then use this to consistently evolve their seed train into the amount of cells the researcher needs. For optimal growth, maintain the same ratio of cells/cm^2 and mL/cm^2 across vessels and reagents by doing the following:

- Apply this formula to calculate your cells by vessel: [(desired cells/cm^2)*(cm^2 of the vessel)].

- Apply this formula to calculate your medium per vessel: [(desired mLs/cm^2)*(cm^2 of the vessel)]. For optimal cell growth and gas diffusion, most applications will require between 0.2 mL and 0.5 mL of medium per cm^2.

Harvesting Your Adherent Cells

You'll know cells are ready to harvest when they appear as a monolayer throughout the culture under the microscope. At that time, scientists will generally remove the cells via chemical or physical means.

Chemical removal via dissociation reagents needs to be optimized for the cell type and application in order to make sure cells are not negatively impacted by the reagent. By contrast, physical removal via cell scrapers may be best for strongly adherent, sensitive cells that might not tolerate dissociation reagents. Also consider your downstream application and how or whether the dissociation reagent may impact your studies.

If you choose to use dissociation reagents, follow this process aseptically using a pipette:

- Remove the media from the flask.

- Add a buffer solution like PBS to the flask to remove any trace amounts of serum that could interfere with the dissociation agent. Gently rock the vessel to even out the solution inside. Soak for 10 to 15 minutes for difficult-to-detach cell lines. Remove the buffer solution.

- Add the dissociation reagent at 0.02 to 0.03 mL/cm^2. Depending on the cell line or dissociation reagent, you may want to incubate the vessel at 37°C to promote dissociation.

- Cells are ready when they appear rounded up but not yet clumped—almost as if you're looking into a night sky filled with thousands of stars. Cloudiness of the liquid is a good sign that they've detached and are ready.

- Dilute the dissociation reagent, typically at a ratio of 1:1 with either the buffer solution or media-contain-

ing serum. Pipette up and down a few times before removing the whole solution and transferring it to the tube.

- Conclude with centrifugation if cells are especially sensitive to the reagent.

Now your cells are ready to count, so you can measure cell density and viability.

Making Cell Culturing Work for You

Adherent cell cultures can open your lab up to a whole new world of experimentation and downstream applications — and it can be fun too. But there can be a lot at stake when you're manipulating living cells, so it's important to do so correctly. By following these essential tips and working your way up to more advanced protocols, you can become a cell culture pro in no time.

Falcon® Cell Culture Products

Falcon cell culture products were the first developed tissue culture treated plasticware that enabled scientists to grow cells *in vitro*. Manufactured from advanced bioanalytical-grade resins and extensively tested, Falcon cultureware ensures high quality and consistent, reliable results.

OUR COMMITMENT TO QUALITY

All Falcon products are manufactured per current Good Manufacturing Practices (cGMP) and ISO 9001 standard. The ISO certification verifies our facilities meet international quality system standards through routine audits, giving our customers an added level of assurance that Corning is committed to superior quality and continuous product improvement.

FALCON CELL CULTURE FLASKS

The vacuum-gas plasma tissue culture treatment provides consistent cell attachment, spreading, and growth. Different growth surfaces are available to meet your individual cell culture requirements, while secure stacking provides stability from accidental tips or spills and protection against contamination.

FALCON CELL CULTURE MULTI-FLASKS

Offer the same footprint and the same reagent volumes and cell seeding densities per unit area as 175 cm^2 flasks. With the same proven surface treatment as all other Falcon flasks, it simplifies scale up.

FALCON CULTURE SLIDES

Allow you to culture cells and then analyze them on a glass microscope slide. Cells are grown in a plastic chamber affixed to a specially prepared glass microscope slide.

FALCON CELL CULTURE DISHES

Have flat, optically clear polystyrene surfaces for distortion-free microscopic visualization of cells and have uniform surface chemistry created by vacuum-gas plasma treatment that promotes cell attachment.

FALCON PLATES

Have a unique labyrinth lid, condensation rings, and deep-well design to control contamination, reduce evaporation, and minimize edge effects, along with a reliable vacuum-gas plasma tissue culture treatment that provides well-to-well and plate-to-plate consistency.

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To learn more about our Falcon cell culture products, visit: **www.corning.com/cellculture**

Cell Culture Schematics and Variables—Helpful Calculations and References

Evolution of cell culturing technology has produced an array of features and options—here we review key concepts and requirements to aid product navigation

Cell culturing is a foundational tool within the life sciences. It plays a critical role in answering basic scientific and translation research questions across disciplines. Within this diverse field, cell culturing technology has evolved across the decades to improve the health and stability of cultures and enable new and specialized applications. The options for culture vessels and accessories can be overwhelming but choosing those best suited for the application can improve yields, help ensure reproducibility, and accelerate workflows, all while saving space in the lab. This article reviews the key considerations and features relating to cell culturing.

Cell growth surface area

Adherent cell cultures are substrate dependent and as such, yields are correlated to surface area. Actual yields depend on the cell line and culture conditions.

Total yields can vary widely by vessel type and geometry and can be further increased through features like ribbing or 3D structures. As such, surface area requirements will be a factor in deciding on the appropriate vessel type. T-flasks, like the ubiquitous T-75, come in rounded and angular shapes and are named by the approximate growth area, e.g. a T-75 is approximately 75 cm^2. Round dishes, like 35 mm and 60 mm dishes, are named for their "style" rather than exact diameters, with growth areas typically ranging 9 cm^2 and to 152 cm^2.

Surface area for microplates is a more complex relationship. As the number of wells on a plate go up, the surface area per well decreases predictably—standard

flat-bottomed 96-well, 384-well, and 1536-well plates have 0.32 cm^2, 0.06 cm^2, and 0.02 cm^2 of surface area per well respectively. The total plate surface areas, however, vary at approximately 30.7, 21.5, and 38.3 cm^2 for standard flat-bottomed 96-well, 384-well, 1536-well plates respectively.

Multilayered flasks offer the most growth area per unit of footprint, like the 1720 cm^2 Corning® HYPERFlask® vessel as well as the 525 cm^2 3-layer and 875 cm^2 5-layer Corning Falcon® Multi-Flasks, all of which have footprints comparable to traditional culture flasks. Such systems offer enhanced scalability for lab processes.

Seeding density matters

Seeding density can affect growth rate and phenotypic expression of the cells within the culture. The optimal seeding density depends on the cell line, media, incubation time, and what condition the cells are in—cryopreserved cells will need higher densities during recovery than an actively proliferating culture.

Seeding density for adherent cells is calculated by cell growth area (cm^2), rather than culture volume (cm^3). Seeding density (D_s) is equal to number of cells (N) divided by growth area (A):

$$D_s = \frac{N}{A}$$

A common starting point for cryopreserved cells is 20,000-25,000 cells/cm^2 to reach confluency in 4-5 days, while an active culture could be halved (12,500 cells/cm^2).

Determine the total volume of media required for the vessel (working volume) to make sure the cells will be covered. The ratio of growth medium to surface area affects the nutrient availability as well as O_2 and CO_2 diffusion. Around 0.2-0.3 mL of medium per cm^2 of surface area is recommended, though more is needed for microwell plates due to the rate of evaporation and meniscus effects. Using more medium will mean less frequent feeding is required but less oxygen will reach the cells.

To work through an example, let's say we want to seed a 96-well plate with freshly thawed cells at a seeding density of 25,000 cells/cm^2 and want to calculate the initial cell suspension concentration required. A flat-bottomed 96-well plate has 0.32 cm^2 growth area per well. To determine how many cells to seed into each well, we multiply 25,000 cells/cm^2 by 0.32 cm^2, for a total of 8,000 cells per well. If we want 100 µl working volume of growth medium per well, we can adjust the initial suspension to 80,000 cells/mL in order to transfer cells and the full medium volume in a single shot.

Whenever changing type or size of culture vessel, consistency in seeding density is key. Adjust the number of cells being seeded by the difference in growth area. To maintain seeding density with changing growth areas, multiply the first seeding density (N_1/A_1) by the second growth area (A_2) to obtain the number of cells required for the second culture.

$$N_2 = \frac{N_1}{A_1} * A_2$$

Surface types—characteristics and function

Cell attachment is a primary concern for adherent cell cultures, and much of the variety in culture vessels on the market relate to attachment surfaces. Cells typically attach best to ionically charged surfaces. Positively charged surfaces attract structural proteins present in serum, which form the best attachment surface for cells. Negatively charged surfaces directly attract attachment proteins within the cell membranes.

Disposable plastic culture vessels have been popular since the 1960's due to difficulties associated with cleaning and attachment in glass. Plasticware today comes in several materials, including polystyrene, polyethylene, polypropylene, polycarbonate, nylon, polytetrafluoroethylene (PTFE), polyvinyl chloride (PVC), cellulose acetate, and polyethylene terephthalate (PET). Each has different physical characteristics, gas permeability, and chemical/reagent compatibilities.

Polystyrene, an early favorite, remains popular for its optical clarity, easy sterilization, and ability to modify it to become more hydrophilic and ionically charged. It is typically treated by oxidation using either gas-plasma or corona discharge, which modifies the long chain polymers. This treatment works best for cultures containing serum.

There are a wide variety of biological materials and synthetic polymers as coatings on the market to enhance attachment in more specialized growth environments. Biological coatings enhance attachment and proliferation but can still present added challenges with batch variability, contaminants, storage requirements, and expense. Poly-D-Lysine coating has been a popular solution, giving the surface a positive charge for improved attachment. It also improves the differentiation of primary cells.

CellBIND® surface from Corning uses a different oxidation technique than the traditional gas-plasma and corona discharge, microwave plasma, that incorporates more oxygen ions into the polystyrene. This improves cell attachment and reduces premature detachment in more difficult culture conditions, including cryopreserved cell recovery.

Other treatments, including Corning® PureCoat™ and Corning Primaria™ surfaces, feature incorporation of both positive and negative functional groups in polystyrene to improve cell adhesion, particularly for primary, transfected, transformed, neuronal, endothelial, and tumor cell types.

Some of the more specialized coatings on the market include the Corning Osteo Assay surface for bone cell assays and Synthemax® self-coating surface for multi-passage expansion of pluripotent stem cells in serum-free media.

To facilitate studies of specific cell functions and performance reliant on three dimensions, new surfaces and scaffoldings have been engineered to provide a more *in vivo*-like substrate, like basement membrane and extracellular matrix structures.

At other end of the spectrum, specialized coatings, like the Corning Ultra-Low Attachment (ULA) surface, have been developed to reduce any adsorption for suspension cultures, neutralizing any attractive forces inherent to polystyrene. These can be particularly helpful for clonal isolation and hybridoma production.

Choosing the right vessel geometry and options for the application

Aside from surface modifications, culture vessels come in a diverse array of shapes and sizes to cover any application or need, such as space saving or automation requirements. Knowing the available options available is key to choosing the best form and geometry for the experimental design or application.

Culture plates are well-suited for optics and can simplify assay and analysis workflows directly within the culture vessel. In addition to clear plates, 96- and 384-well plates are available in black to improve fluorescence imaging and white to improve luminescence imaging. Wells come in flat-bottomed formats in different sizes, including "half area" wells, as well as round bottomed and V-bottomed formats. Specialized options exist for 3D spheroid cultures with optically clear bottoms and low attachment surfaces, including the Corning® Elplasia® plates for high-throughput applications.

Adherent cell cultures can be suspended to allow for basolateral feeding in an *in vivo*-like environment using Transwell® cell culture inserts, which are permeable supports that fit inside plates. These inserts come with different pore sizes, membranes, and coatings or treatments to suit varied experimental requirements. For example, Corning Matrigel® matrix for invasion assays is used to coat some polycarbonate or PET membrane inserts, and transparent collagen treated PTFE membrane promotes attachment and spreading. Further specialty options facilitate automation, use in diffusion chambers, or tissue and organ growth and development.

Dishes are also well suited for optics and can come with vents for gas exchange and stacking rings for easier han-

dling. Specialized dishes for IVF culture provide a center 1-3 mL well and an outer moat to maintain humidity.

Flasks are an ever-popular option that come in an array of shapes to suit preference or application and improve ease-of-use. Some flask shapes, like U-shaped flasks with rounded corners or those with modified angles and canted necks improve tool access for scraping cells and pipetting. Canted neck flasks also improve pouring.

Many flask design options improve space efficiency. Angled neck and straight neck flasks offer the full bottom area for cell growth. Low profile and stacked or multi-layer flasks are space saving solutions particularly useful for restricted incubator space. These options generally offer greater surface area within the same footprint of traditional flasks and are stackable.

Flask caps similarly vary for preference and application. Plug seal caps are liquid- and gas-tight, one-piece designs intended for closed systems. Phenolic style caps are designed for gas exchange when loosened and are suited for open systems. Vent caps have 0.2 µm pore, non-wettable (hydrophobic) membrane in the cap for reducing spills and sterile gas exchange even after contact with media.

Surface Area of Culture Vessels[1]

			Single Well Only						Entire plate		
		Format	Well Diameter (Bottom) (mm)	Approx. Growth Area (cm^2)	Seed Cell #*	Average Cell Yield**	Total Well Volume (µL)	Working Volume (µL)	Approx. Growth Area (cm^2)	Average Cell Yield**	Working Volume (mL)
Microplates	96-Well Microplates	**Flat Bottom**	6.4	0.32	8×10^3	3.2×10^4	360	100 - 200	30.7	3.07×10^6	9.6-19.2
		Round Bottom	6.4	Dependent on medium volume			330	100 - 200	Dependent on medium volume		9.6-19.2
		V-Bottom	6.4	0.38	9.5×10^3	3.8×10^4	320	100 - 200	36.5	3.65×10^6	9.6-19.2
		Half Area	4.5	0.16	4×10^3	1.6×10^4	190	50 - 100	15.4	1.54×10^6	4.8-9.6
	384-Well Microplates	**Standard (square wells)**	2.7	0.056	1.4×10^3	5.6×10^3	112	25 - 50	21.5	2.15×10^6	9.6-19.2
		Low Volume	2	0.031	775	3.1×10^3	50	5 - 40	12	1.2×10^6	1.9-15.3
	1536-Well Microplates	**Low Volume**	1.2	0.011	275	1.2×10^3	2.3	1 - 1.5			
		Clear Flat Bottom (square wells)	1.63	0.025	625	2.5×10^3	12.5	08-May	38.3	3.8×10^6	7.7-15.4
		Solid Flat Bottom (square wells)	1.53	0.023	575	2.3×10^3	12.5	08-May	35.3	3.5×10^6	7.7-15.4
Multi-well plates	Multi-well plates	**6-well**	34.8	9.5	2.375×10^5	9.5×10^5	16.8	1,900 - 2,900	57	5.7×10^6	11.4-17.1
		12-well	22.1	3.8	9.5×10^4	3.8×10^5	6.9	760 - 1,140	45.6	4.56×10^6	9.1-13.7
		24-well	15.6	1.9	4.75×10^4	1.9×10^5	3.4	380 -570	45.6	4.56×10^6	9.1-13.7
		48-well	11	0.95	2.375×10^4	9.5×10^4	1.6	190 -285	45.6	38.4×10^6	9.1-13.7
								Volume per Well	**Volume for Inside of Insert (µL)**		
	Transwell® Permeable Insert Supports	**6-well**	24 mm	4.67	1.168×10^5	4.67×10^5		2,600	1,500		
		12-well	12 mm	1.12	2.8×10^4	1.12×10^5		1,500	500		
		24-well	6.5 mm	0.33	8.25×10^3	3.3×10^4		600	100		
		96-well	4.26 mm	0.143	3.575×10^4	1.4×10^4		235	75		
		100 mm dish	75 mm	44	1.1×10^6	4.4×10^6		1,300	9,000		

Surface Area of Culture Vessels[1] Cont.

	Format	Approx. Growth Area (cm^2)	Seed Cell #*	Average Cell Yield**	Recommended Medium Volume (mL)	Max working volume (mL)
Dishes	35 mm	9	2.25×10^5	9.0×10^5	1.8 - 2.7	
	60 mm	21	5.25×10^5	2.1×10^6	4.2 - 6.3	
	100 mm	55	1.375×10^6	5.5×10^6	11 - 16.5	
	150 mm	152	3.8×10^6	1.52×10^7	30.4 - 45.6	
	245 mm (square)	500	1.25×10^7	5.0×10^7	100 - 150	
Flasks	25 cm^2	25	6.25×10^5	2.5×10^6	5 - 7.5	10
	75 cm^2	75	1.875×10^6	7.5×10^6	15 - 22.5	60-90
	Low profile flask	100	2.5×10^6	1.0×10^7	20-30	40
	150 cm^2	150	3.75×10^6	1.5×10^7	30 - 45	210
	175 cm^2	175	4.375×10^6	1.75×10^7	35 - 52.5	250
	225 cm^2	225	5.625×10^6	2.25×10^7	45 - 67.5	370
	Expanded surface flask	235	5.875×10^6	2.35×10^7	47-70.5	250
	HYPERFlask® vessel	1,720	4.3×10^7	1.72×10^8	560 - 565	565

*Seeding number calculated based on 25,000 cells per cm^2 seeding density. Optimal seeding density varies.

**Average cell yield is based on 1x 105 cells/cm^2 at confluency

SOURCE:

1. Corning. (2021) Corning Cell Culture Product Selection Guide. https://www.corning.com/catalog/cls/documents/selection-guides/CLS-CC-010.pdf.

A Flask for Any Task

A wide variety of culture vessels from start to scale

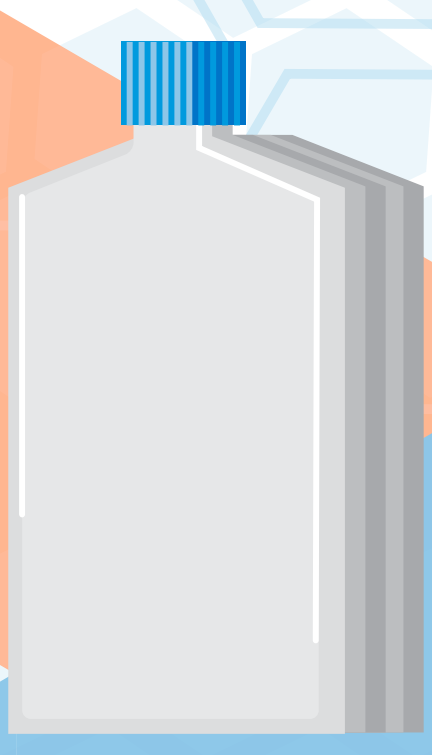
Whatever vessel sizes your research requires, Corning's got you covered. Our flask selection takes your lab through every stage of benchtop scale-up — and all the milestones in between.

CORNING® HYPERFLASK® CELL CULTURE VESSELS

10X the growth area of a 175 cm^2 flask with the same footprint, gas-permeable film for efficient gas exchange and cell growth, and automation capability.

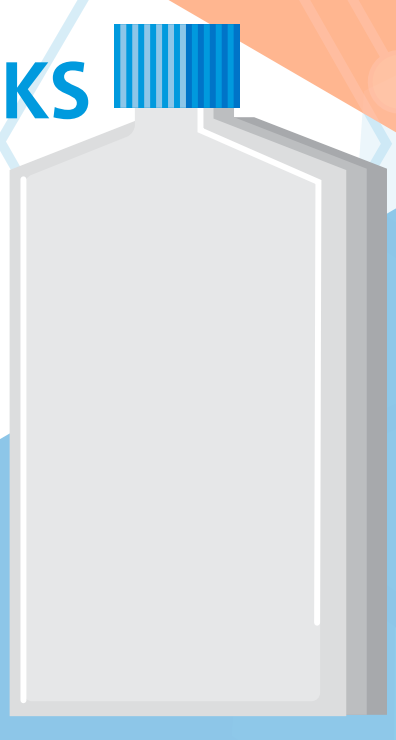
1,720 cm^2
Corning HYPERFlask Cell Culture Vessel

560-565 mL Recommended Working Volume
2.5 x 10^8 Average Cell Yield*



FALCON® MULTI-FLASKS

Achieve scale and save time with these high-performers that pack in more surface area with a smaller footprint.

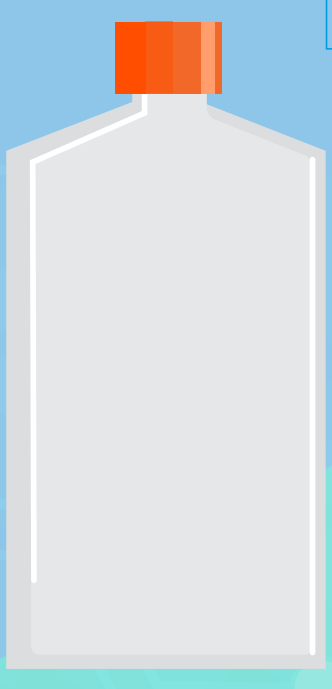


875 cm^2
Falcon 5-layer Multi-Flask

25-50 mL Recommended Working Volume per Layer

525 cm^2
Falcon 3-layer Multi-Flask

25-50 mL Recommended Working Volume per Layer



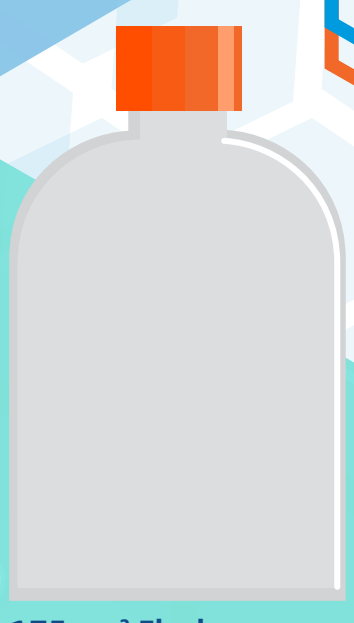
225 cm^2 Flask

45-67.5 mL Recommended Working Volume
2.25 x 10^7 Average Cell Yield*

Options for:
Single-layer Flasks
Falcon Multi-Flasks
Corning HYPERFlask Vessels

Pick Your Treatment
(available for selected flasks):

- Non-treated
- Tissue Culture-treated
- Corning CellBIND® Surface
- Ultra-Low Attachment



175 cm^2 Flask

35-52.5 mL Recommended Working Volume
1.75 x 10^7 Average Cell Yield*

Single-layer Flask Options

Pick Your Cap

- Phenolic
- Plug Seal
- Vent

Pick Your Style

- Traditional
- Rectangular
- U-Shape
- Canted Neck
- Straight Neck

150 cm^2 Flask

30-45 mL Recommended Working Volume
1.5 x 10^7 Average Cell Yield*

75 cm^2 Flask

15-22.5 mL Recommended Working Volume
7.5 x 10^6 Average Cell Yield*

25 cm^2 Flask

5-7.5 mL Recommended Working Volume
2.5 x 10^6 Average Cell Yield*

12.5 cm^2 Falcon Flask

25 mL Recommended Working Volume

SINGLE-LAYER FLASKS

With a variety of sizes, treatments, and caps, these non-pyrogenic vessels are a benchtop essential.

Keep your cells happy and healthy, no matter how many you're growing.

Find the right flask for your research at:

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**Assumes an average yield of 1 x 10^5 cells/cm^2 from a 100% confluent culture. Yields from many cell types can be lower than this.*

Cryopreservation of Cells Guide

Cell cryopreservation puts your cell lines in suspended animation, effectively stopping biological time. While it might seem the stuff of science fiction, cryopreservation is a fundamental process in cell culturing, a meticulous process that requires the utmost precision and care.

So how does the cryopreservation of cells work? Let's break this multistep process down.

Step 1: Select the cells

Optimal cryopreservation occurs when cells are in the best possible condition and near the end of the logarithmic growth phase. Carefully examine your cultures for signs of microbial contamination. Growing cultures for several passages in an antibiotic-free medium before testing can make contaminants that might have gone undetected reach a more detectable level.

Examine your samples under a microscope, then test them by direct culture for bacteria, yeasts, fungi, and mycoplasmas. Mycoplasmas present unique issues because they can sneak through tests; you'll need to test culture stocks again after they've been frozen.

Step 2: Harvest the cells

Harvest the cells using the proper procedure for the cell type—and be as gentle as possible. Once you've harvested the cells, wash off or inactivate any dissociating agents, which can damage the cells. Use a centrifuge only if you must but be gentle—only use a force hard enough to yield a soft pellet.

Pool the contents of the harvested culture vessels to ensure the uniformity of the final frozen stock. Dilute or concentrate the cell suspension as needed to achieve twice the desired concentration.

Keep the cells chilled to slow cell metabolism and to prevent clumping. Cells can be gassed with carbon dioxide when necessary to prevent alkaline pH shifts.

Step 3: Store the cells

Selecting the right cryoprotective agents prevents—or at least minimizes—cell damage during cryopreservation. Choosing the right storage vessel is also critical. Choosing the wrong one carries numerous risks, including injury, damage to vessels, and contamination or loss of frozen stock. Heat-sealable glass ampules and polypropylene screw-capped vials (internal or external thread) are the most commonly used vessels for cryogenic storage, but sealing issues with the former have researchers and industry professionals increasingly preferring the latter.

Step 4: Cool the cells

The cooling rate must be uninterrupted, and it must be slow enough to afford the cells time to dehydrate but fast enough to prevent damage from dehydration. For most animal cell cultures, the ideal cooling rate is a steady drop between 1°C and 3°C per minute. Larger or less permeable cells might need to cool more slowly because they take longer to dehydrate.

Some labs use programmable electronic cooling units, which provide precise control of the freezing process and yield uniform, reproducible results. Others use mechanical units that offer sufficient control of the process at a less expensive price. One of the most economical and common methods for cell lines is the use of ultracold freezers with insulated polystyrene foam boxes.

Step 5: Store the cells

Once the stock is frozen, you need to move fast. Use an insulated container filled with dry ice or liquid nitrogen to transfer the frozen stock to permanent storage. Speed is the key to avoid warming the vials and damaging the cells.

Most cell culture labs use liquid nitrogen freezers, but the most important feature in any permanent frozen storage location is that it can reliably maintain temperatures below -130°C. Even a temporary rise in temperature can damage the cells.

Step 6: Thaw the cells

Cooling cells must be done gradually, but the opposite is true when thawing cells. Rapid thawing reduces the formation of damage-causing ice crystals within the cells as they rehydrate. Place your container in warm water and stir it gently until it's completely thawed. For most cell cultures, thawing for 60 to 90 seconds at 37°C achieves the best results.

Step 7: Let the cells recover

Remove the cryoprotective agents from the cells as quickly and gently as possible to avoid the damage that prolonged exposure to these agents can cause. How you remove the cryoprotective agents depends on the type of agent and the type of cells—cells that are sensitive to cryoprotective agents, for example, require gentle centrifugation to remove the agent. When glycerol is the cryoprotectant, the sudden addition of a large volume of fresh medium to the thawed cell suspension can damage or destroy the cells. To avoid this, take the cells through several stepwise dilutions with an equal volume of warm medium every 10 minutes before further processing to give the cells time to adjust.

In general, most cells recover normally if the cryoprotective agent is removed through a medium change within six to eight hours of thawing.

When successfully preserved, frozen cells need little maintenance and can be a lifeline if you lose cell cultures to contamination or accident. Frozen cell cultures are especially useful for long-term experiments, as their suspended animation ensures that biological variants are kept to a minimum.

Survival of the Coolest.

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Corning® CoolCell® provides alcohol-free freezing at a rate of -1°C/min. Ideal for cell cryopreservation.

- Rapidly returns to room temperature when removed from the freezer
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- Ease of use
- Simple, consistent way to standardize controlled-rate freezing

Corning CoolCell Container		Conventional Isopropanol (IPA) Containers
10-15 minutes	**Time-saving** Time needed before container can be reused	60 minutes
NO	**Operating Cost**	YES
No ongoing operating cost		Operating cost for IPA purchase
-80°C	**Easier Handling**	
Non-stick when taken out of -80°C freezer; spill-, leak-, break-proof when dropped		High risk of spills, leaks, breaks if dropped
	Less Effort	
No need to record the the number of freezing cycles		Requires costly alcohol replacements every 5 uses

For more specific information on claims, visit www.corning.com/certificates.

Warranty/Disclaimer: Unless otherwise specified, all products are for research use or general laboratory use only. Not intended for use in diagnostic or therapeutic procedures. Corning Life Sciences makes no claims regarding the performance of these products for clinical or diagnostic applications.

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5 Things to Consider When Customizing Cell Culture Media (and Other Reagents)

Regardless of your lab's size or scale, there will come a time when you need a customized solution for your media, sera, or reagents. Off-the-shelf lab products are great for many production needs, but scientists often find themselves searching for tailor-made options when the standard catalog doesn't quite fit the project.

Scenarios where customized solutions offer the most benefit run the gamut across research areas, whether developing novel formulations for cell culture media, rinses, and cryopreservation solutions or fulfilling exact-volume packaging for fill tolerance control.

Given the flexibility of their applications, custom solutions can meet a range of research and production needs in areas like tissue processing and regenerative medicine, vaccine manufacturing, and downstream purification. But customization isn't only ideal for specialized disciplines. Increasingly, many labs are realizing the value that custom products can bring to day-to-day operations.

But there's a catch—with customization comes many considerations. Researchers who thoughtfully explore each one will be best equipped to reap the benefits of customization with minimal risk, cost, and resources.

Here's what you should consider as you contemplate your options for bespoke lab products.

1. Going in-house comes with costs and headaches.

You might have already toyed around with the idea of going the DIY route — particularly if you're a smaller, nimbler lab. But that option comes with its own hassles, says Andria Pate Collier, a senior product line manager of media at Corning Life Sciences.

"You don't see the cost structure of using your own folks to manufacture something, but that doesn't mean it doesn't cost you in the long run," Collier said. "Research-

ers may go in thinking that it's cheaper to manage it internally, but then suddenly it gets out of control when you're making different things from different areas within the labs."

In-house formulations may save money at the outset, but it leaves little leeway to consolidate the costs of raw materials as workflows get more specific and fragmented — and that's on top of having to ensure current good manufacturing practice compliance. As you become more reliant on patchwork processes, you might lose out on the purchasing power, regulatory know-how, and supply chain resilience that an outsourced outfit like Corning can offer.

2. Formulation isn't the only thing that can be customized.

Many researchers require a novel formulation of cell culture media or other solutions, but those aren't the only components that can be tailor-made. Even standard products can be customized by putting them in different packaging.

Buying supplies such as large batches of rinse water or custom-filled volumes of catalog media in made-to-order packaging can cut costs while reducing waste and the risk of measuring errors.

"Often, labs have very specific requirements around volumes," Collier said. "Maybe you need 1.1 liters of media and you want it really precise when it comes to that fill volume tolerance because you might be adding something else to that media before you use it. You'd want to be sure that, every time, you have exactly the volume you need. It's much easier to make that align with the workflow if every bottle is prefilled to a designated amount."

Additionally, some suppliers — including Corning — can produce and white-label custom media, an offering worth considering if you're looking to sell your formulations.

"Custom solutions shouldn't be limited to something you use internally," Collier said. "You could always explore a contract manufacturing relationship with your supplier, where you buy a customized product, have the supplier apply your label to the product, and then resell it."

3. You'll need a plan.

Depending on your need, a customized solution will require some amount of lead time — anywhere from a few weeks for small-scale products to many months for a full cGMP-ready run. Connect with your supplier well in advance to discuss your needs and timing expectations.

"With Corning, most small-size orders can be fulfilled within three to four weeks," said Amyntrah Maxwell, an assistant product line manager of custom media at Corning Life Sciences. "But when you move toward larger needs, you'll want to start the process about 12 weeks out."

Advanced or nuanced projects could have even longer lead times, Collier adds.

"Sometimes, customers might want to start working with us six or eight months out," she said. "Suppliers will typically have a lot of questions, and there's a great deal of interaction and regular meetings to make sure everything is right before submitting the final purchasing order."

4. Customization isn't just for big or well-funded labs.

Cost was once a barrier for customized solutions, but Maxwell says that notion is no longer true. With more pilot and small-batch options, modestly sized operations, such as academic labs and small biotech companies, can take advantage of made-to-order media.

"People are starting to realize it's an affordable option," Maxwell said. "You can find budget-conscious options for whatever batch size you need, regardless of whether you have plans to scale up. Now, you don't have to buy

a thousand liters to test out a custom formula. You can buy them in 100, 20, or even 5 liters to start."

When comparing suppliers for small-scale batches, ask whether a supplier will provide free consultation services. Corning, for example, offers complimentary customization services to every customer.

5. Ensure that your supply chain can withstand disruptions.

As manufacturers shifted priorities to focus on customers developing treatments and vaccines for COVID-19, many supply chains struggled under the challenges of the pandemic. If you're considering a customized solution from a third-party provider, ask how they respond to market disruptions and how they'll keep your orders on track.

"We've seen a lot of customers come to us because some of their previous manufacturers no longer have the capacity to service their needs," Collier said, adding that Corning's supply chain was built with resilience in mind by diversifying its partner base and anticipating market shortages. "It has pushed a lot of the smaller customers out, as well as those who work in animal health."

Making Customization Work for You

Customization offers something for everyone — but you'll need to be diligent when designing yours so that you don't overlook key considerations that can cost you in the long run. Factor in these practices, and you'll be well on your way to sourcing the ready-made solution best for you.

Corning is ready, willing, and able to provide you quality serum and custom media – available when and where you need it – to help you produce consistent, repeatable results. Talk to our media and serum specialists now.

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As a laboratory scientist, you never stop thinking about where your research is headed or if there's a better way to get there. At Corning, we get it. That's why we offer you a comprehensive portfolio of vessels, advanced surfaces, and cell culture media and work with you one-on-one to help you make the most of Corning products—from start-up to scale-up.

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