Biocompare
The Buyer's Guide for Life Scientists

CELL CULTURE FUNDAMENTALS

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Why Culture from a Single Cell?

Benefits include uniform populations to observe, experiment on, and learn from.

Cell cultures derived from a single cell are clonal. They are composed of cells with identical genetic makeup having been grown under identical conditions, thereby affording a homogenous population for a host of downstream applications such as the production of monoclonal antibodies.

Single cell-derived clonal cultures can be obtained from primary cells or established cell lines that have been adapted to culture conditions.

But in general, the only cells that survive as a single cell long enough to form a colony or spheroid are stem cells and cancer cells. Other cell types don't have that ability, and will typically die or cease to reproduce when isolated.

Given the appropriate treatment, stem cells can either be maintained in their stem-like state, or driven to differentiate into one or another more differentiated cell types. Stem cells and cancer cells can be allowed to proliferate in culture, to be used as platforms for drug testing, for example, or other downstream assays.

What follows are some tips for culturing single cells.

Easy does it

The first step in making a single cell culture is, of course, to generate single cells. Whether from cultured cells or tissue, the process should be done gently and carefully to keep the cells healthy.

Various treatments, including several different animal-derived and recombinant enzyme formulations (with or without the use of a chelating agent such as EDTA), as well as some newer non-enzymatic methods, can release cells from a substrate and dissociate them into single cells. It may be important to optimize the process—tissues often require manual manipulation, and different cell types may require a stronger treatment, or a longer period of time, while others may be more sensitive. If possible, do a test run on a tissue or bulk culture to make sure that most cells go in to a single-cell suspension, that individual cells are viable, and that adherent cells are still able to attach to a surface after being dissociated.

Isolating the cells

Among the most important considerations for obtaining single cells for culture is the way in which

they are isolated and divided into individual compartments. The choice will largely depend upon several factors, including downstream application, desired throughput, equipment available and training of the personnel doing the isolation, whether a rare cell or all cells in a population is desired, whether the cells are to be selected based upon molecular markers, and generally the sort of parameters that should be optimized for.

The simplest method of individuating cells for culture is perhaps serial dilution. In its simplest version, a suspension of cells is placed in a well of a 96-well plate; half is removed and moved to a second (medium-containing) well; half of that is moved to the next well; and so on, iteratively diluting the suspension until there is on average, one cell per well. A closely related technique is to calculate the dilution factor such that, when aliquoted, each well has a high likelihood of containing one and only one cell.

Certain cultures, such as induced pluripotent stem cells (iPSCs), arise from single cells that grow into clonal colonies. These can be picked using techniques such as micro-manipulation, in which the colony is manually cut out from the culture. Similarly, laser microdissection has been used to isolate colonies as well. These are time- and labor-intensive techniques that require a lot of training.

More automated, higher-throughput methods include using an instrument like a flow cytometer to sort cells directly into plates or tubes. One of the advantages of such an approach is that cells can be directly chosen based on the presence or absence of molecular markers, allowing rare cells to be found. Flow cytometry can subject the cells to high pressures that can impact viability, though. Recently, some lower pressure, microfluidic-based cell sorters/dispensers with rudimentary fluorescence sorting capabilities have come on the market.

Attachment issues

Perhaps the most difficult part of culturing single stem or cancer cells is getting them to attach to the new surface. Once they do, they will generally grow under the same conditions as a bulk culture.

It's important to think about the substrate—the vessel, and especially the coating—they will attach to. Typically, adherent cells will be plated in wells with a tissue culture (TC)-treated surface. The polystyrene surface is modified to carry a net negative charge, making it more hydrophilic and allowing for better attachment. For cells that are still reluctant to attach, other surface treatments, such as Corning's CellBIND® surface, offer an even more negatively charged surface. Vessels are also available with a wide variety of surface treatments and coatings, including biological and mimetic extracellular matrix (ECM) proteins, to afford a more *in vivo*-like environment. A cell culture surface selection guide can be found **here**.

Starting with a low volume of medium—especially when using a cell sorter—will allow the cell to fall to the substrate quicker. The plate should be pre-filled with medium and allowed to equilibrate in an incubator (being wary of evaporation) until immediately before sorting. Given that cells are so sensitive in a single cell environment, starting the attachment process under ideal temperature and gas conditions that much sooner may make them that much more successful in taking hold.

Culture medium and supplements

Once they attach and are settled in, single cells should thrive in the medium in which bulk cultures are grown. To get to that point, the best place to start is with the published literature, colleagues, and vendors, as well as perhaps a little trial-and-error.

It is often necessary to adjust the concentration of **fetal bovine serum** (FBS), for example, or of supplements such as growth factors, in order to get the cells over that initial hump.

Conditioned medium (CM) —supernatant from cell culture—has been used on some occasions to provide the necessary growth conditions for the cells. However, CM can introduce variables with unknown consequences.

It may be better not to use antibiotics in the initial culture as well, if possible, to minimize the stress on the sensitive single cells.

Conclusion

Many factors contribute to obtaining and culturing single cells, from isolation methods to selecting substrate, medium, and supplements. When done successfully, it offers uniform populations to observe, experiment on, and learn from.

To view Corning's full portfolio or cell culture solutions visit **corning.com/cellculture**.

Why Primary Cells?

The rewards of a culture more like its origin are worth the extra effort.

Roughly speaking, a "primary cell" is one that has just been removed from a host and placed into culture. Compared to continuous (transformed) cell lines, such cultures are not immortal, will not proliferate indefinity, and are more challenging to maintain.

Yet primary cells can represent a more physiological model, allowing researchers to garner information not available from cell lines—which are susceptible to genetic drift and its consequent abnormalities—or *in vivo* models. Primary neurons are used for brain research, while primary keratinocytes are used as a skin models for toxicology and wound healing. Primary cells can be made from healthy or diseased, the young or aged, male or female, European or African, human tissue. They allow comparisons of physiology or biochemistry, development, disease or aging, or the effects of drugs and toxins, while minimizing the need to experiment on animals. They are sources for cell and transplant therapy.

Tissue to culture

In its outline, setting up a primary culture from solid tissue is fairly straightforward. For most cell types, once harvested and decontaminated, the tissue is separated into individual cells. This typically begins with cutting and mincing to obtain small pieces. Pieces are then treated enzymatically and/or mechanically to dissociate cells from connective tissue. The resulting mix can be further broken down by trituration and micro-filtration, punctuated by a series of washes, centrifugation, and re-suspension. The mostly single-cell population is plated and allowed to grow.

Some cell types are relatively hearty, while others require more care. As with many things, there is no one-size-fits-all, and it is recommended that a researcher checks with colleagues and the literature for the best ways to handle a particular tissue.

For example, which dissociation enzyme(s) will strike the best balance between freeing the cells from their extracellular matrix and inflicting harm? Similarly with mechanical methods—is a standard mortar and pestle sufficient, or is some more specialized instrument called for? Or would a chemical method, such as cation chelation by EDTA—generally slower but gentler—be enough?

The substrate

While cultured cell lines have been adapted to grow on plastic, primary cells are coming directly from a tissue with an *in vivo* chemical and mechanical environment very different from plastic. In some cases,

primary cells have difficulty attaching to a standard tissue culture (TC)-treated plate and require a different substrate that better recapitulates at least some of the properties they are accustomed to.

A variety of surface-enhanced cultureware is available in different formats, including multiwell plates, T-flasks, and dishes. Corning's CellBIND® surface, for example, is more hydrophilic than standard TC-treated plates, improving the ability of some fastidious cell types to attach and grow.

Sometimes cells require a biological substrate to which to attach, or researchers are aiming to emulate a more physiological milieu. For these situations, cultureware is available pre-coated with Corning® Matrigel® matrix or extracellular matrix (ECM) derived proteins such as laminin, collagen, or fibronectin, as well as recombinant proteins. Researchers can also purchase soluble coating formulations, allowing them to vary the concentrations as well as specific mixtures to their needs.

For a detailed guide to vessels, surfaces, coatings, and their applications, see **Corning's Cell Culture Product Selection Guide** and **Corning's Cell Culture Surfaces Guide.**

The medium

Primary cell cultures are almost always a mixture of the cell types found in the tissue, each with their own requirements. When a particular cell type is desired over others, it may be useful to alter the culture conditions to favor the desired cell type, allowing it to out-compete the others. Researchers may also want to drive a cell type to differentiate in the culture.

Established cell lines can often make do with a basal medium formulation supplemented by bovine calf serum or fetal bovine serum (FBS). But this may not be enough to allow many primary cell types to thrive.

Primary cells may have very specialized and nuanced requirements, including a host of specific growth factors and supplements, to favor proliferation of specific cell types or guide them to differentiate.

Keeping the culture safe

Contamination carried over from the harvesting process can be an issue in primary cultures. Of course, all standard tissue culture precautions—such as using disposable vessels and tools, autoclaving or filtering reagents, proper use of a laminar flow hood, and the like—should be observed. And while the use of antibiotics is discouraged for long-term cultures for various reasons, this is not the case for the first week or two while the culture is being established.

For more on preventing contamination, download **Corning's Guide to Understanding and Managing Cell Culture Contamination**.

Once established, the primary culture will not go on forever: it is subject to the Hayflick Limit of 20–60 doublings (not passages!) before a cell senesces. If this particular culture is important and not feasible to replace—because it was derived from a unique patient, for example, or it is part of a longer-term project —cells should be frozen down early on. For a guide to cryopreservation, please see **Corning's Guide for Cryogenically Storing Cell Cultures**.

It is also possible that some cells in the culture will become immortal and surpass the Hayflick Limit. These can then be selected for and grown from single cells to form a clonal cell line. If the cell line is to be of use, it is important at this stage to determine whether the primary culture's properties of interest have been retained.

Keep it real, or not

Researchers often want the most realistic environment for their primary cells, allowing them to best recapitulate *in vivo* interactions and functionality. One trend in the field is to use specialized cultureware, or natural hydrogels, inducing the cells to form spheroid structures or otherwise grow out in three dimensions.

At the same time, researchers may want a simpler, less variable system with fewer biological components.

The aim then is to strike a balance. Especially for stem cells, this may mean replacing a traditional feeder layer with Corning® Matrigel® matrix, or even using mimetic surface treatments, medium and supplements, to further control for the inherent variability of biological components.

Primary cells can be challenging, but the rewards of a culture more like its origin can be more than worth it.

Maintaining Sample Temperature

Q&A with a Corning scientist

Generally speaking, bad things can happen when a cell sample temperature gets too hot or cold. Temperature instability can affect many downstream variables in any given project, from limiting the functionality of reagents or other experimental agents to eventually killing good cells. These impacts can delay timelines, increase costs, and diminish data integrity.

Despite the importance of consistent temperature regulation in the lab, sometimes sample viability can become a saga of **"survival of the coolest."** But when cultures rely on outside cooling mechanisms like electricity or ice—or live in a busy environment with a lot of people flowing in and out—those survival odds can take an unfortunate hit.

So, what can scientists do about this issue? We sat down with Corning Scientific Support Manager Kyung-A "Katie" Song, Ph.D., to get her expertise on controlling temperature in a lab.

Q: How does temperature affect cell viability?

Every type of cell has a predetermined optimal temperature for attachment and preparation. If a cell cannot be cultured at that optimal temperature, it can't be maintained in a healthy state and will automatically die.

However, it's not just cell viability that's at stake here. Reagents, enzymes, and other experimental carriers are also very sensitive to stable temperature conditions. With improper regulation, not only can cells die, but reagents and enzymes can be similarly affected and rendered unusable. This drives up costs.

So, you can see why temperature regulation is so important. Without it, an experimental result can be unreliable or unpredictable—or your time, effort, and costs can be negatively affected.

Q: Is overcooling as big a risk as overheating?

Yes. We commonly think about overheating because the cells are composed of a protein that generally cannot withstand high temperatures, but overcooling can also be dangerous to cells. For example, at subzero temperatures (Celsius), ice crystals begin to form inside the cell, which changes the concentration of the culture medium. As a result, water begins to move out of the cell, initiating dehydration and shrinking, and leading to cell damage and death.

Q: Why is temperature so tricky to control in the lab setting?

Lab environments usually need a range of constant, stable temperatures for many different applications. For example, Eukaryotic cell culture conditions usu-

ally need to be at 37 degrees Celsius with carbon dioxide gas, but reagents and enzymes should be stored at -20 or -80 degrees Celsius.

So, to address this variety of temperature demands, a lab will typically have different refrigerators, freezers, incubators, water baths, heat blocks, and other tools. All of these mechanisms are susceptible to problems and need regular inspection, maintenance, and monitoring. They're also usually dependent on electricity, which can be problematic during utility blackouts.

Aside from these factors, labs can also be busy, shared spaces with many people coming in and out. So even with the best equipment, it's challenging to maintain a stable temperature when you have a door that's constantly opening and closing.

Q: What are the known problems with traditional temperature regulation equipment?

There are certainly challenges to having battery- or electric-dependent appliances or other tools that need frequent monitoring and replacement, which can be expensive, time-consuming, and labor-intensive.

Similarly, containers that are dependent on ice for cooling can require frequent ice changes and trips to the ice machine, and there's always a contamination risk when melted ice seeps into vials. Ice cooling can also impact temperature stability. When you measure the temperature of a plate directly on ice, you'll find that the temperature in each well isn't uniform.

There are also challenges associated with the conventional isopropanol container, or IPA—affectionately known as Mr. Frosty. IPAs are used in many labs for cryogenic freezing, but they come with several disadvantages, such as the need to check and refill the isopropanol after every five uses. Also, alcohol degradation can lead to temperature variability, and the screw cap can be difficult to remove when frozen.

Q: What are some good investments to consider when shopping for cooling tools?

Good choices include systems that don't have as many of the dependencies and disadvantages you see from ice baths, IPAs, or battery-powered or electricity-dependent appliances. It's also smart to ensure that tools are portable. Our alcohol-free system, **Corning® CoolCell® container**, is one option. This container provides a consistent cooling profile without the downsides of a traditional IPA container and without ongoing operating costs.

Similarly, we also have the **Corning CoolRack® module** as a temperature stabilization barrier for tubes placed in ice baths. This product is made of proprietary thermal conductive materials which reduce the variability that comes from tubes or wells being placed directly on ice or other temperature sources. As another option that doesn't depend on ice, the **Corning CoolBox™ module** enables prolonged cooling for up to 16 hours or freezing for up to eight hours with one Corning XT cooling core.

Q: What else should scientists know about sample temperature control in the lab?

It may seem like a basic thing, but the principles of temperature regulation are very important and worth revisiting for scientists of all backgrounds and experience levels. Very often when you're troubleshooting, the key to success comes down to addressing temperature variability—whether the focus is on sample temperature control or regulation of reagents and enzymes. Even small things can make a big difference.

What's Killing My Cell Cultures?

Troubleshooting cell growth issues

Nothing's more maddening than cells that won't grow or cell cultures that won't attach. Stunted cell growth and erratic culture behavior can delay projects, frustrate teams, and leave researchers wondering what went wrong and how they can fix it.

Solutions to these problems aren't always straightforward. Cell culturing is complex, and it takes time to pick apart the process and isolate issues. Generally, the most common issues include abnormal growth patterns or spotty, uneven, or inconsistent cell attachment. Other problems include slow or sudden changes in growth rate or unexplainable outcomes.

Such issues tend to be associated with culture technique, incubation, and media. Let's explore a few of these pain points and how you can preempt their effects with good practices.

Check the technique

When something goes wrong with cell cultures, the first thing to assess is your handling techniques. For example, feeding cultures can expose them to contamination, a common and serious problem. Incorrect mixing and pipetting can also cause issues:

- **Uneven handling or insufficient mixing** of the cell inoculum can cause foam or bubbles, which can hinder cell growth and attachment and leave spots on vessels. Pour or pipet media carefully to ensure bubble-free material.
- **Static electricity** can disrupt cell attachment in plastic vessels, especially in low-humidity areas. Try not to rub the vessels against the package when opening them. Wiping the outside of the vessel or using an antistatic device can also stymie static.
- **Insufficient cell inoculum** can cause heavier growth on the sides of vessels. So can using too little medium when refeeding cultures.
- **Spin speeds** of roller bottles can cause heavy growth or clumping. Set your starting speed between half a revolution per minute and one revolution per minute and modify as needed. Keep rollers clean to prevent slips or stalls.

You can usually tell that something's gone awry when you see uneven or abnormal patterns of cell attachment or growth. To best detect these kinds of problems, fix sample cultures using glutaraldehyde or ethanol, then stain them before observation.

Incubation issues

Even though the point of using an incubator is to minimize environmental factors like variations in temperature, many variants can still affect growth rate and culture viability:

- **Temperature variations** happen when the incubator is repeatedly opened during the experiment; how and where vessels are stacked can also cause temperature variations. Don't open the incubator unless you have to and put mission-critical cultures in the back. Consider, too, the positional effects of stacking dishes; the lowest dishes are closest to the metal shelf, so they heat faster.
- **Evaporation** affects cell growth rates and patterns; minimize it by keeping water reservoirs full and by humidifying incoming gases with a **gas-washing cylinder**.
- **Vibration** can cause unusual cell growth, such as concentric cell rings. Vibrations can come from a loose fan motor in the incubator or from any number of sources, such as foot traffic or motorized appliances. Keep incubators on sturdy surfaces that don't have any other pieces of equipment that vibrate.

Other environmental precautions, such as keeping incubators level and without toxic impurities, are also essential to proper growth.

Blame the media

Media defects aren't always visible, and many factors—such as insufficient reagent quality, buffers, and filtration, and even fluorescent light toxicity—affect how media function.

Outside of ensuring that you're using the suitable formulation for your research and purchasing high-quality supplies, the best way to test media function is to experiment. Compare the troublesome media with the same media from another manufacturer. If the new media yields the same result, you might have technique or incubation issues.

Think like a scientist

If your technique and treatment aren't right, your live cell cultures will suffer. While it can be time-consuming to isolate variables one by one, doing so often delivers the best insight about what went wrong and why.

Approach abnormal cell growth as you would with any other research problem: Hypothesize, test, and analyze. Before long, you'll figure out how to keep those precious cells alive—and your research on track.

Get more cell culture troubleshooting tips in the **Corning Guide for Identifying and Correcting Common Cell Growth Problems.**

Cell Contamination, Containment, and Correction

Tips on good cell culture practices

Cell contamination is a major concern in 3D cell culture labs as well as with more traditional 2D monolayer cultures. If biological contaminants are undetected, they can significantly affect your resulting data, rendering it inaccurate or useless, or make any products manufactured by the cultures unusable.

How do you devise cell culture protocols that **prevent contamination?** While contamination can be controlled, it cannot be totally eliminated. So, what do you do when contamination inevitably happens?

What are the causes of cell contamination?

The major causes of contamination in the cell culture system are typically either chemical or biological. In 3D cell culture, contamination can occur in various components, such as the bio-ink or the **extracellular matrix**.

Chemical contamination

Chemical contamination is the presence of a nonliving substance that may cause unwanted effects on the cell culture. Most chemical contaminants are found in the cell culture media and originate from either reagents, the water used to make the reagents, or additives to supplement reagents.

Chemical contaminants may include metal ions, impurities, or endotoxins in media, sera, and/or water; impurities in gases used in CO_2 incubators; plasticizers used in tubing and bottles; free radicals in media generated by photoactivation of various components; residue from disinfectants or chemicals used to clean equipment; or deposits left on instruments, glassware, or other equipment.

Biological contamination

Biological contaminants fall into two categories: those that are easy to detect (such as bacteria, molds, and yeast) and those that are difficult to detect (such as viruses, protozoa, mycoplasmas, other cell lines, and insects).

Bacteria, molds, and yeast are the most common contaminants in cell culture environments. They are readily detected when antibiotics are not present, but when antibiotics are used routinely in cell culture protocols, it can cause a slow-growing infection that is difficult to detect. By the time this type of contaminant is discovered, it may have already compromised the cell culture.

Contamination prevention in the lab

Biological contaminants often enter cell cultures by use of nonsterile supplies and media. Sterilization procedures using autoclaving or dry heat sterilization must be followed properly.

Ensure proper maintenance and operation of sterilization autoclaves and ovens. Don't overpack the autoclave, which can cause uneven heating and thus fail to sterilize the entire contents of the autoclave. Be sure to use a long enough sterilization cycle for the materials and liquids you are sterilizing—often, a viscous fluid or large volume of liquid is understerilized, which can lead to contamination. To maintain sterility, store the supplies and solutions properly in a dust- and insect-free area.

Using proper aseptic technique is also important, of course. Review the **top 10 ways to reduce contamination risk** in the lab. Airborne particle risk can be minimized by washing hands, donning clean gloves and lab coats. Minimize antibiotic use. Work with one cell line at a time. Make sure to monitor routinely for contamination.

What are proper post-contaminant procedures?

When you've discovered contamination, autoclaving is recommended to cure the contaminated culture and prevent it from spreading to other cultures. Occasionally, you may need to attempt to save a valuable culture that has become contaminated. This is usually only tried with mycoplasma infections, as fungus and yeast contaminants are unlikely to be eradicated, bacterial contaminants are often already antibiotic-resistant, and many cultures lose critical characteristics after cleanup. Mycoplasma contamination is most often treated with antibiotics, but no approach is totally successful at eradication. What's more, treatment does not typically eliminate infection completely, but pushes it to below-detectable levels. Over time, the surviving mycoplasma can recover and develop into a more serious contamination problem.

Understanding cell culture contamination best practices ensures that your research quality does not have to suffer from an unexpected occurrence, whether big or small. The most important takeaway, however? Prevention is indeed the best medicine.

Resources

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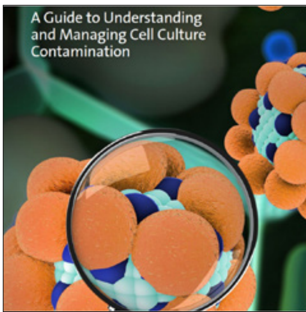
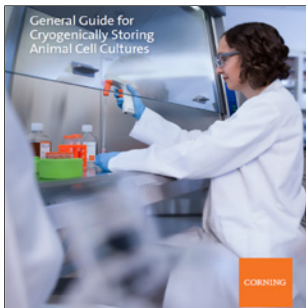


Guide to Understanding and Managing Cell Culture Contamination

Infographic: Top 10 Ways to Reduce Contamination Risk



General Guide for Cryogenically Storing Animal Cell Cultures

A Guide for Identifying and Correcting Common Cell Growth Problems with Adherent Cells

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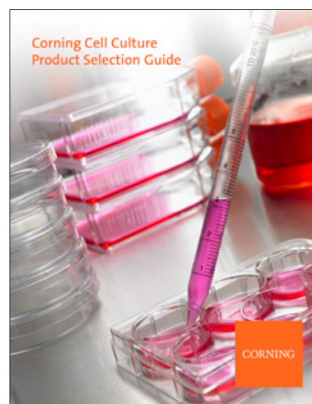

Corning Cell Culture Product Selection Guide

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