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Introductory Guide to Cell Culture Basics

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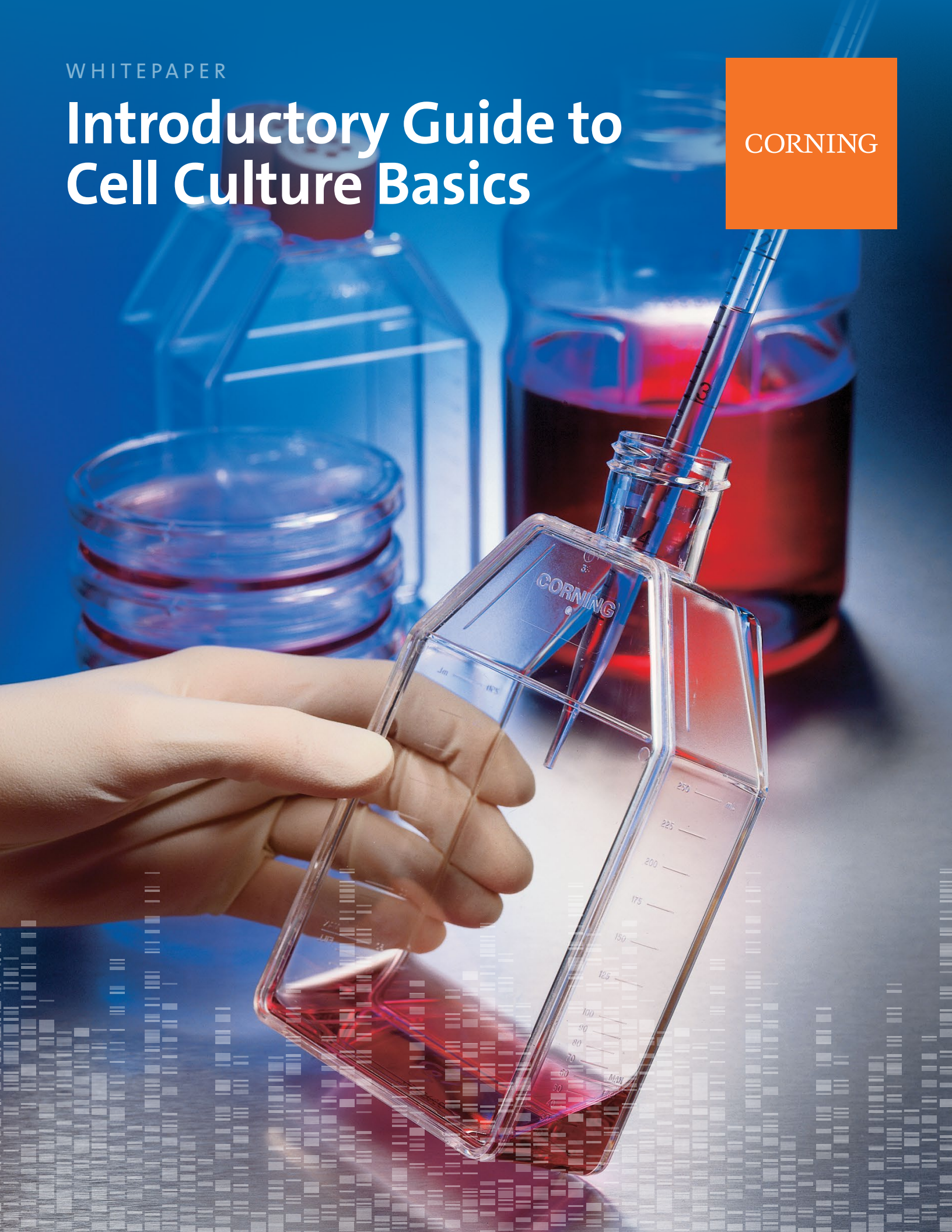

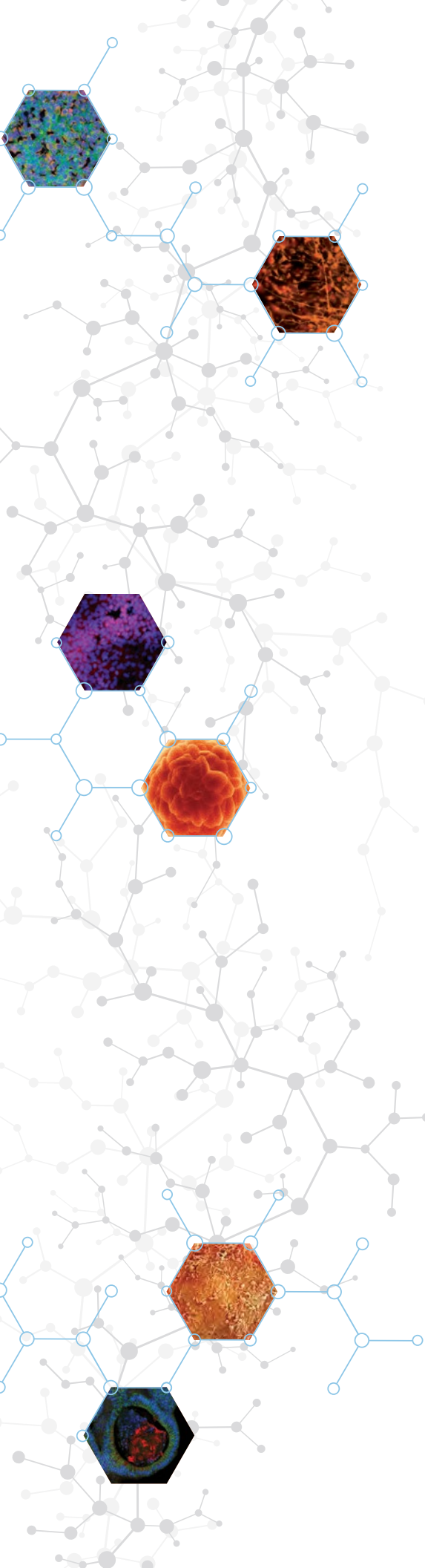

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Cell culture is the foundation for many different production and research applications. Culturing cells in controlled conditions gives laboratories insight into tumor development and progression, cell signaling mechanisms, drug sensitivity in specific tissues, and more.

Molecular laboratories, cell-based production facilities, and primary research labs all use cell culture, with the basics leading to advanced work with spheroids, organoids, and other multi-cell tissue models.

What Is Cell Culture?

Cell culture is the fundamental technique used to grow cells *in vitro*, usually in a dish, flask, or other vessel suitable for maintaining optimal conditions. Under controlled conditions, cell lines can be maintained at optimal growth for days or weeks, with the opportunity for harvesting, scaling up, and storage.

Researchers utilize cell culture as a powerful tool in cytogenetic, biochemical, and molecular labs in various diagnostic and research studies. These include disease modeling, toxicity testing, cancer research, virology, cell-based manufacturing, genetic engineering, and gene therapy. Cell culture also figures prominently in the pharmaceutical industry for drug discovery, screening, and development.

History of Cell Culture

Following the invention of the microscope, scientists have known that tissue is made up of cells. Early attempts at growing these cells outside the body succeeded in the hanging drop experiments of Ross Granville Harrison in 1907. Using an inverted microscope slide and cover slip assembly, he successfully maintained frog embryo tissue *in vitro*, using the technique to demonstrate nerve fiber development. Harrison also set the foundation for an aseptic technique that remains essential for a successful culture to this day.

In the late 1940s, researchers started to establish cell lines. Rather than harvest each culture afresh from mammalian tissues, they developed conditions and harvested mostly from tumors for infinite growth of a single cell type. As this method became more popular, commercialization in the 1970s provided standardized growth media, culture vessels, and technological advancement in the field.

How Do Cells Grow in Culture?

Cells grow successfully in culture when researchers carefully control the growth environment. This means controlling factors such as pH, humidity, and temperature — in addition to providing nutrients for optimal growth and maintaining a sterile environment free of **contamination**.

Primary Cells vs. Cell Lines

Two types of cells are used in cell culture: primary cells and cell lines.

Primary cells are isolated from a source — either from a tumor or a tissue donor. These are disrupted and then seeded into a growth medium. Primary cultures have a limited lifespan but show normal morphology compared to the tissue of origin. They may contain a mix of different cell types reflective of the tissue of origin. Primary cultures also show low mutation rates but are extremely sensitive to media conditions.

In contrast, cell lines are usually derived from cancer tissue or are primary cells that have been transformed using a viral oncogene, for example. This means they're considered immortalized and will continue growing and dividing indefinitely. Compared to tissue-derived cells, they often exhibit abnormal morphology and mutate readily. However, researchers can also handle them easily, and they grow well in culture.

Primary cells can be transformed for immortalization using a viral oncogene. However, using already immortalized cell lines makes it easy to scale up for production, research, drug discovery, and so on, ensuring consistency and decreased variability.

Adherent vs. Suspension Culture

Cell culture growth can either be adherent, where the cells grow attached to the culture vessel surface, or suspension, where the cells are free-floating in the culture medium. You can more easily feed and monitor adherent cells, as you can observe growth directly by using a microscope for the degree of confluence over the vessel surface. Suspension cultures are harder to feed and grow, and checking on them is more difficult. To monitor growth, you need to count them rather than directly observe them. Each method requires different culture vessels for success.

Culture Medium

The culture medium provides nutrients and other conditions necessary for successful cell growth. For *in vitro* growth, cells need amino acids, sugars, vitamins, glutamine, salts, and minerals in the medium surrounding them. The culture medium often contains other factors, such as medications, to control contaminating bacteria and buffering to maintain a steady pH and osmolality.

Additives such as phenol red are useful as visual indicators or alerts for changes in pH. Many growth media also include serum, such as fetal bovine serum, plus antibiotics and growth factors specific to culture requirements.

Different cell lines require different media and additives for success, which is where a cell culture Corning representative can help.

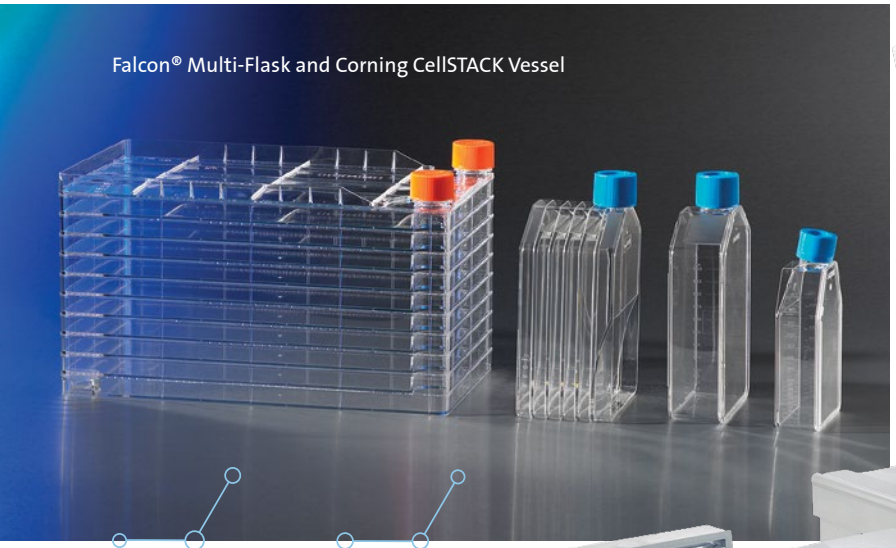
What Is the Cell Culture Process?

To grow cells *in vitro*, you must first isolate and digest them from tumor or human/animal tissue as primary cells or suspended from stock as cell lines, then seed them onto a culture plate or vessel. The vessel you choose depends on both the cell type and the total cell yields you desire. This also influences the medium you use, additives, growth specifications and confluency, and laboratory facilities.

Choosing Cultureware

The best vessel for maximum optimal cell growth depends heavily on factors such as cell attachment issues, product yield requirements, equipment and space availability, and technical skills and operator experience in the laboratory.

Adherent cells need suitable surfaces to grow on. These are ones that promote adhesion and provide a large enough surface area that exposes the growing cells to the culture medium for nutrition and pH buffering. Types of vessels for adherent cells will be a variety size of T-flask, multi-flask, roller bottle, **Corning® HYPERFlask® Vessel**, **Corning CellSTACK® Vessel**, **Corning HYPERStack® Vessel**, and **Corning CellCube® System**. Suspension cell cultureware will be Erlenmeyer flasks, spinner flasks, cell expansion bags, and rocker bags, with the latter two allowing efficient scale-up.



Falcon® Multi-Flask and Corning CellSTACK Vessel

Corning HYPERStack Vessel

Corning CellCube System

Scaling up Production

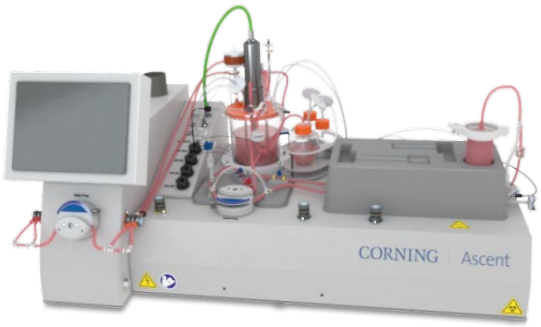
Scaling up production is often possible without increasing the footprint. Layering technology using permeable growth surfaces, such as with Corning® CellSTACK® Vessel or the **High Yield PERformance (HYPER) range of products**, multiplies the growth surface area for massive scale-up. This technology maintains the product's consistency, which, coupled with ease of use, gives much more cell surface area per footprint at greater efficiencies.

Fixed bed reactor (FBR) technology vastly increases scale within a small footprint, with savings in terms of consistency in cell growth and cellular product quality. The **Corning Ascent® FBR system** combines the benefits of adherent platforms with the scale and automation of suspension manufacturing systems. The bioreactor's stacked woven mesh disks enable uniform media flow, cell adhesion, and growth within a scalable platform.

Using Appropriate Vessels for Enhanced Culture

Factors that optimize cell growth include attachment treatments to encourage, or sometimes discourage, cell attachment on growth surfaces. Most flasks are untreated polystyrene, which isn't always optimal for some cell lines.

Modifications or coatings make surfaces more hydrophilic to encourage cell attachment and promote cell growth. These coatings include **extracellular matrix** and **Corning BioCoat® treatments** with collagen, gelatin, and fibronectin, for example. **Advanced options** include synthetic, animal-free cultureware, and Ultra-Low Attachment (ULA) surface options.



Corning Ascent FBR System

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How Do You Set Up a Cell Culture Lab?

Critical factors for success start with providing an aseptic environment for cell manipulation, storage, and optimal growth conditions.

- **A clean bench with a laminar flow hood** creates an aseptic environment, and high-efficiency particulate absorbing (HEPA)-filtered air and ultraviolet light keep the environment aseptic. Consumables entering the hood must be sterile. All work done here uses an aseptic technique to reduce contamination.
- **A cell culture incubator** provides a controlled growth environment, maintaining a constant temperature and humidity for growth. This is usually 37°C with 5 percent CO_2 for mammalian cells or 26°C to 30°C for insect cells. High humidity is important for reducing evaporation from the medium, so the incubator usually has a water tray at the bottom. Keep the tray sterile and topped up.
- **Tools** are important and often depend on techniques and personal preference. A pipet controller for consistent handling and filling can prevent operator fatigue in repetitive tasks. Store serological pipets and tips in aseptic boxes prior to use at the clean bench.
- **Cell storage** requires low temperatures; labs require vapor phase liquid nitrogen tanks (highly recommended over direct liquid nitrogen) and the cryogenic vials that go into them. For long-term storage, put the cells into cryogenic vials overnight at -80°C and then transfer during vapor phase into a liquid nitrogen storage tank.

How Do You Culture Cells?

Cell culture relies on four basic steps for maintenance, growing cells for research, production processes, and other purposes:

1. Thaw cells from storage.
2. Seed, feed, and passage cells.
3. Count cells (for assay and analysis).
4. Freeze cells for short-/long-term storage.

Thawing

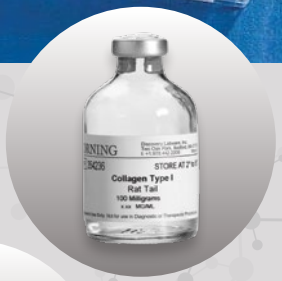
Cells must be thawed gently from storage in the vapor phase of liquid nitrogen.

1. Take the cryogenic vial from the storage tank, and immediately place it in a 37°C water bath.
2. Thaw for less than one minute, then gently add a warmed complete medium into the vial.
3. Use a centrifuge to spin down and concentrate the cells in a pellet.
4. Carefully aspirate the supernatant, and wash the cell pellet using a washing buffer. This step also removes the freezing media, which typically contains cryoprotective agents (CPA) such as dimethly sulfoxide (DMSO) or glycerol. In particular, DMSO is toxic if the cells are exposed at room temperature for prolonged periods.
5. Resuspend the cells using the complete culture medium, then seed them into a suitable culture vessel.

Passaging

Cells in culture require changes in medium to support optimal growth. Eventually, the cells multiply so much that they become confluent or tightly packed together. When this happens, you need to split and passage them to avoid mutation, stress, or change in morphology. The passage number is the number of times cells have been split. Frequency usually reflects the cell line doubling time.

You can usually assess confluence visually, using direct microscopy for adherent cells. It's also indicated by a phenol red color change. It's best to avoid the culture becoming too confluent as cells may be changed irreparably. Aim to passage cells at moderate to less than 85-95 percent confluency when there's still space to grow.



Passaging Adherent Cells

1. Aspirate the old medium. Wash with washing buffer, and add a dissociation buffer to disrupt the cells from the growth surface.
2. Incubate to detach the cells (you may need to tap the flask), and then inactivate the dissociation buffer using two to three times the volume of the complete cell culture medium.
3. Spin down to the pellet, and collect the cells.
4. Resuspend the cells in the growth medium, and plate them out at the required seeding density (cells per cm^2).
5. Label according to cell name, researcher name, medium, passage date, and number.

Passaging Suspension Cells

Concentrate the cells by spinning them down—you don't need a dissociation medium. Collect the pellet, then resuspend and plate out at the required seeding density (cells per mL).

Refer to best practices for seeding density; your supplier can help with this information.

Plating and Counting Cells

For research studies, the proper plate will be determined by how many cells will be used and their application. Count cells in suspension in a hemocytometer or by automated **cell counting** to find the number of cells per milliliter.

Freezing Cells

For optimal storage that preserves cell viability, freeze the cells in a protective medium. A freezing medium contains a high concentration of serum and DMSO as a cryoprotectant. Cooling rate is challenging during the freezing process. The cryoprotectant helps to prevent intracellularice formation and avoid serious dehydration effects.

Collect cells from the culture and count for density, then spin down to the pellet.

1. Resuspend the pellet in a freezing medium to achieve the correct density for storage using cryogenic vials.
2. Store the vials at -80°C for one day, then place them into the vapor phase liquid nitrogen. For research studies, the proper plate will be determined by how many cells will be used and their application.

Summary

Cell culture is a powerful technique and it's the foundation for a variety of research studies. Understanding the cell types and cultureware available and learning the techniques and best practices required will lead to success. Primary papers are often useful, but remember that your vendor is also a valuable resource. Please visit the Corning **technical support pages** for assistance and information.

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