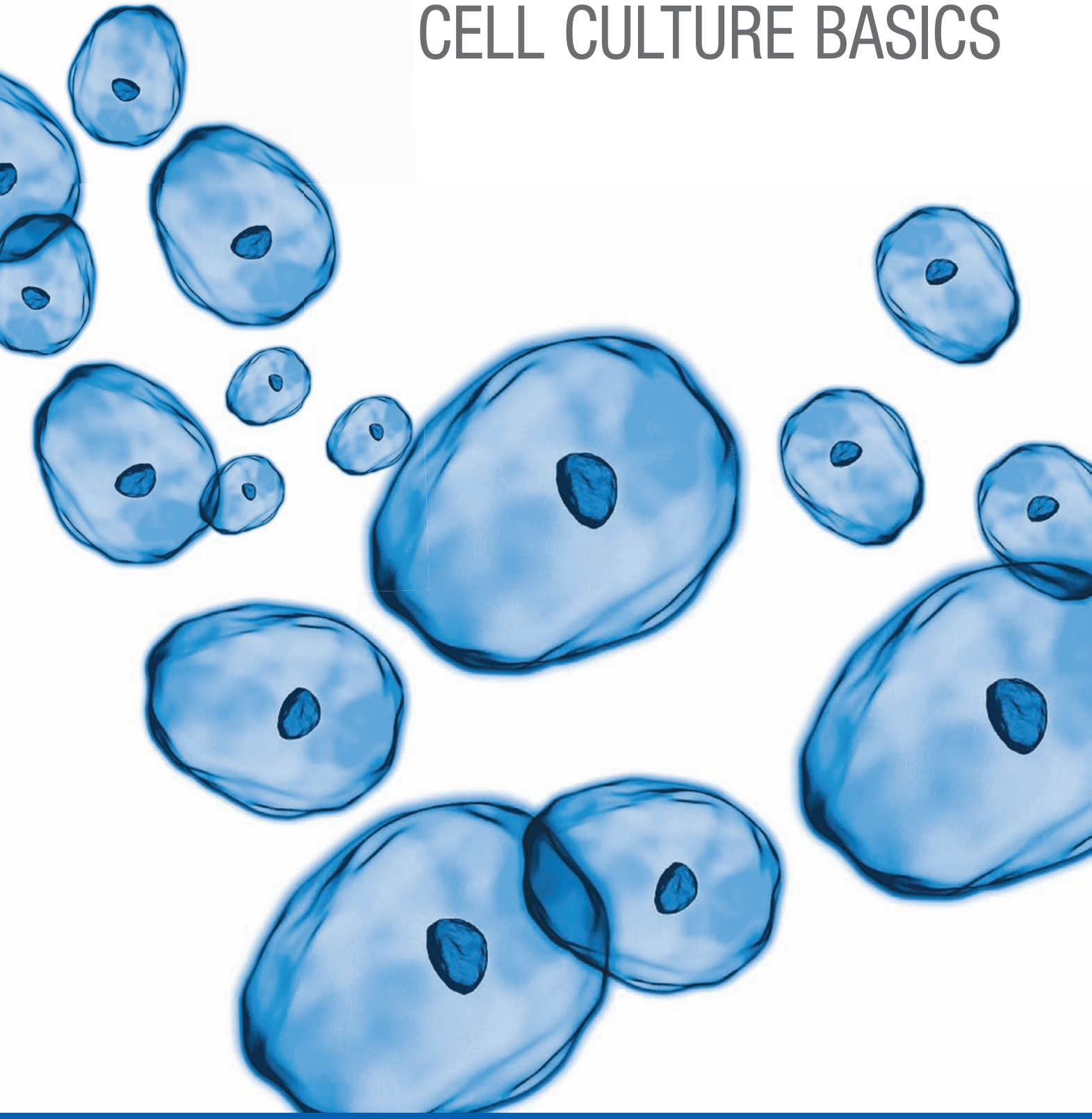
GIBCO®

invitrogen™

CELL CULTURE BASICS



Handbook

Contents

Introduction

Purpose of the Handbook

Cell Culture Basics Companion Handbook is a supplement to the Cell Culture Basics instructional videos available online at www.invitrogen.com/cellculturebasics.

The handbook and videos are intended as an introduction to cell culture basics, covering topics such as getting familiar with the requirements of a laboratory dedicated to cell culture experiments, laboratory safety, aseptic technique, and microbial contamination of cell cultures, as well as providing basic methods for passaging, freezing, and thawing cultured cells.

The information and guidelines presented in the handbook and the instructional videos focus on cell lines (finite or continuous) and omit experiments and techniques concerning primary cultures such as isolating and disaggregating tissues.

Note that while the basics of cell culture experiments share certain similarities, cell culture conditions vary widely for each cell type. Deviating from the culture conditions required for a particular cell type can result in different phenotypes being expressed; we therefore recommend that you familiarize yourself with your cell line of interest, and closely follow the instructions provided with each product you are using in your experiments.

Introduction to Cell Culture

What is Cell Culture?

Cell culture refers to the removal of cells from an animal or plant and their subsequent growth in a favorable artificial environment. The cells may be removed from the tissue directly and disaggregated by enzymatic or mechanical means before cultivation, or they may be derived from a cell line or cell strain that has already been already established.

Primary Culture

Primary culture refers to the stage of the culture after the cells are isolated from the tissue and proliferated under the appropriate conditions until they occupy all of the available substrate (i.e., reach **confluence**). At this stage, the cells have to be **subcultured** (i.e., passaged) by transferring them to a new vessel with fresh growth medium to provide more room for continued growth.

Cell Line

After the first subculture, the primary culture becomes known as a **cell line** or **subclone**. Cell lines derived from primary cultures have a limited life span (i.e., they are **finite**; see below), and as they are passaged, cells with the highest growth capacity predominate, resulting in a degree of genotypic and phenotypic uniformity in the population.

Cell Strain

If a subpopulation of a cell line is positively selected from the culture by cloning or some other method, this cell line becomes a **cell strain**. A cell strain often acquires additional genetic changes subsequent to the initiation of the parent line.

Finite vs Continuous Cell Line

Normal cells usually divide only a limited number of times before losing their ability to proliferate, which is a genetically determined event known as **senescence**; these cell lines are known as **finite**. However, some cell lines become immortal through a process called **transformation**, which can occur spontaneously or can be chemically or virally induced. When a finite cell line undergoes transformation and acquires the ability to divide indefinitely, it becomes a **continuous cell line**.

Culture Conditions

Culture conditions vary widely for each cell type, but the artificial environment in which the cells are cultured invariably consists of a suitable vessel containing a substrate or medium that supplies the essential nutrients (amino acids, carbohydrates, vitamins, minerals), growth factors, hormones, and gases (O_2, CO_2), and regulates the physico-chemical environment (pH, osmotic pressure, temperature). Most cells are **anchorage-dependent** and must be cultured while attached to a solid or semi-solid substrate (**adherent** or **monolayer culture**), while others can be grown floating in the culture medium (**suspension culture**).

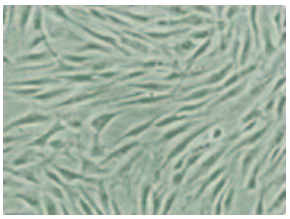
Cryopreservation

If a surplus of cells are available from subculturing, they should be treated with the appropriate protective agent (e.g., DMSO or glycerol) and stored at temperatures below –130°C (**cryopreservation**) until they are needed. For more information on subculturing and cryopreserving cells, refer to the **Guidelines for Maintaining Cultured Cells**, page page 26–page page 39.

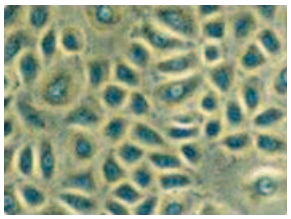
Morphology of Cells in Culture

Cells in culture can be divided in to three basic categories based on their shape and appearance (i.e., **morphology**).

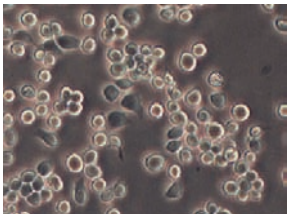
- **Fibroblastic** (or fibroblast-like) cells are bipolar or multipolar, have elongated shapes, and grow attached to a substrate.



- **Epithelial-like** cells are polygonal in shape with more regular dimensions, and grow attached to a substrate in discrete patches.



- **Lymphoblast-like** cells are spherical in shape and usually grown in suspension without attaching to a surface.



Applications of Cell Culture

Cell culture is one of the major tools used in cellular and molecular biology, providing excellent model systems for studying the normal physiology and biochemistry of cells (e.g., metabolic studies, aging), the effects of drugs and toxic compounds on the cells, and mutagenesis and carcinogenesis. It is also used in drug screening and development, and large scale manufacturing of biological compounds (e.g., vaccines, therapeutic proteins). The major advantage of using cell culture for any of the these applications is the consistency and reproducibility of results that can be obtained from using a batch of clonal cells.

Cell Culture Laboratory

Safety

In addition to the safety risks common to most everyday work places such as electrical and fire hazards, a cell culture laboratory has a number of specific hazards associated with handling and manipulating human or animal cells and tissues, as well as toxic, corrosive, or mutagenic solvents and reagents. The most common of these hazards are accidental punctures with syringe needles or other contaminated sharps, spills and splashes onto skin and mucous membranes, ingestion through mouth pipetting, and inhalation exposures to infectious aerosols.

The fundamental objective of any biosafety program is to reduce or eliminate exposure of laboratory workers and the outside environment to potentially harmful biological agents. **The most important element of safety in a cell culture laboratory is the strict adherence to standard microbiological practices and techniques.**

Biosafety Levels

The regulations and recommendations for biosafety in the United States are contained in the document *Biosafety in Microbiological and Biomedical Laboratories*, prepared by the Centers for Disease Control (CDC) and the National Institutes of Health (NIH), and published by the U.S. Department of Health and Human Services. The document defines four ascending levels of containment, referred to as biosafety levels 1 through 4, and describes the microbiological practices, safety equipment, and facility safeguards for the corresponding level of risk associated with handling a particular agent.

Biosafety Level 1 (BSL-1)

BSL-1 is the basic level of protection common to most research and clinical laboratories, and is appropriate for agents that are not known to cause disease in normal, healthy humans.

Biosafety Level 2 (BSL-2)

BSL-2 is appropriate for moderate-risk agents known to cause human disease of varying severity by ingestion or through percutaneous or mucous membrane exposure. Most cell culture labs should be at least BSL-2, but the exact requirements depend upon the cell line used and the type of work conducted.

Biosafety Level 3 (BSL-3)

BSL-3 is appropriate for indigenous or exotic agents with a known potential for aerosol transmission, and for agents that may cause serious and potentially lethal infections.

Biosafety Level 4 (BSL-4)

BSL-4 is appropriate for exotic agents that pose a high individual risk of life-threatening disease by infectious aerosols and for which no treatment is available. These agents are restricted to high containment laboratories.

For more information about the biosafety level guidelines, refer to *Biosafety in Microbiological and Biomedical Laboratories, 5th Edition*, which is available for downloading at www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.

Safety Data Sheet (SDS)

Safety Data Sheet (SDS), also referred to as Material Safety Data Sheet (MSDS), is a form containing information regarding the properties of a particular substance. The SDS includes physical data such as melting point, boiling point, and flash point, information on the substance's toxicity, reactivity, health effects, storage, and disposal, as well as recommended protective equipment and procedures for handling spills.

The SDSs for all Invitrogen products are available at www.invitrogen.com/sds.

Safety Equipment

Safety equipment in a cell culture laboratory includes **primary barriers** such as biosafety cabinets, enclosed containers, and other engineering controls designed to remove or minimize exposure to hazardous materials, as well as **personal protective equipment (PPE)** that is often used in conjunction with the primary barriers. The **biosafety cabinet** (i.e., cell culture hood) is the most important equipment to provide containment of infectious splashes or aerosols generated by many microbiological procedures as well as to prevent contamination of your own cell culture. For more information, see **Cell Culture Hood**, page 7.

Personal Protective Equipment (PPE)

Personal protective equipment (PPE) form an immediate barrier between the personnel and the hazardous agent, and they include items for personal protection such as gloves, laboratory coats and gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. They are often used in combination with biosafety cabinets and other devices that contain the agents or materials being handled. We recommend that you consult your institution's guidelines for the appropriate use of PPE in your laboratory.

Safe Laboratory Practices

The following recommendations are simply guidelines for safe laboratory practices, and they should not be interpreted as a complete code of practice. Consult your institution's safety committee and follow local rules and regulations pertaining to laboratory safety.

For more information on standard microbiological practices and for specific biosafety level guidelines, refer to *Biosafety in Microbiological and Biomedical Laboratories, 5th Edition* at www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.

- Always wear appropriate personal protective equipment. Change gloves when contaminated, and dispose of used gloves with other contaminated laboratory waste.
- Wash your hands after working with potentially hazardous materials and before leaving the laboratory.
- Do not eat, drink, smoke, handle contact lenses, apply cosmetics, or store food for human consumption in the laboratory.
- Follow the institutional policies regarding safe handling of sharps (i.e., needles, scalpels, pipettes, and broken glassware).
- Take care to minimize the creation of aerosols and/or splashes.
- Decontaminate all work surfaces before and after your experiments, and immediately after any spill or splash of potentially infectious material with an appropriate disinfectant. Clean laboratory equipment routinely, even if it is not contaminated.
- Decontaminate all potentially infectious materials before disposal.
- Report any incidents that may result in exposure to infectious materials to appropriate personnel (e.g., laboratory supervisor, safety officer).

Cell Culture Equipment

The specific requirements of a cell culture laboratory depend mainly on the type of research conducted; for example, the needs of mammalian cell culture laboratory specializing in cancer research is quite different from that of an insect cell culture laboratory that focuses on protein expression. However, all cell culture laboratories have the common requirement of being free from pathogenic microorganisms (i.e., asepsis), and share some of the same basic equipment that is essential for culturing cells.

This section lists the equipment and supplies common to most cell culture laboratories, as well as beneficial equipment that allows the work to be performed more efficiently or accurately, or permits wider range of assays and analyses. Note that this list is not all inclusive; the requirements for any cell culture laboratory depend the type of work conducted.

Basic Equipment

- Cell culture hood (i.e., laminar-flow hood or biosafety cabinet)
- Incubator (humid CO_2 incubator recommended)
- Water bath
- Centrifuge
- Refrigerator and freezer (–20°C)
- Cell counter (e.g., Countess® Automated Cell Counter or hemacytometer)
- Inverted microscope
- Liquid nitrogen (N_2) freezer or cryostorage container
- Sterilizer (i.e., autoclave)

Expanded Equipment

- Aspiration pump (peristaltic or vacuum)
- pH meter
- Confocal microscope
- Flow cytometer

Additional Supplies

- Cell culture vessels (e.g., flasks, Petri dishes, roller bottles, multi-well plates)
- Pipettes and pipettors
- Syringes and needles
- Waste containers
- Media, sera, and reagents
- Cells

Cell Culture Laboratory

Aseptic Work Area

The major requirement of a cell culture laboratory is the need to maintain an aseptic work area that is restricted to cell culture work. Although a separate tissue culture room is preferred, a designated cell culture area within a larger laboratory can still be used fort sterile handling, incubation, and storage of cell cultures, reagents, and media. The simplest and most economical way to provide aseptic conditions is to use a **cell culture hood** (i.e., biosafety cabinet).

Cell Culture Hood

The cell culture hood provides an aseptic work area while allowing the containment of infectious splashes or aerosols generated by many microbiological procedures. Three kinds of cell culture hoods, designated as Class I, II and III, have been developed to meet varying research and clinical needs.

Classes of Cell Culture Hoods

Class I cell culture hoods offer significant levels of protection to laboratory personnel and to the environment when used with good microbiological techniques, but they do not provide cultures protection from contamination. They are similar in design and air flow characteristics to chemical fume hoods.

Class II cell culture hoods are designed for work involving BSL-1, 2, and 3 materials, and they also provide an aseptic environment necessary for cell culture experiments. A Class II biosafety cabinet should be used for handling potentially hazardous materials (e.g., primate-derived cultures, virally infected cultures, radioisotopes, carcinogenic or toxic reagents).

Class III biosafety cabinets are gas-tight, and they provide the highest attainable level of protection to personnel and the environment. A Class III biosafety cabinet is required for work involving known human pathogens and other BSL-4 materials.

Air-Flow Characteristics of Cell Culture Hoods

Cell culture hoods protect the working environment from dust and other airborn contaminants by maintaining a constant, unidirectional flow of **HEPA-filtered air** over the work area. The flow can be **horizontal**, blowing parallel to the work surface, or it can be **vertical**, blowing from the top of the cabinet onto the work surface.

Depending on its design, a **horizontal flow hood** provides protection to the culture (if the air flowing towards the user) or to the user (if the air is drawn in through the front of the cabinet by negative air pressure inside). **Vertical flow hoods**, on the other hand, provide significant protection to the user and the cell culture.

Clean Benches

Horizontal laminar flow or vertical laminar flow "clean benches" are **not** biosafety cabinets; these pieces of equipment discharge HEPA-filtered air from the back of the cabinet across the work surface toward the user, and they may expose the user to potentially hazardous materials. These devices only provide product protection. Clean benches can be used for certain clean activities, such as the dust-free assembly of sterile equipment or electronic devices, and they should never be used when handling cell culture materials or drug formulations, or when manipulating potentially infectious materials.

For more information on the selection, installation, and use of biosafety cabinets, refer to *Biosafety in Microbiological and Biomedical Laboratories, 5th Edition*, which is available for downloading at www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.

Cell Culture Hood Layout

A cell culture hood should be large enough to be used by one person at a time, be easily cleanable inside and outside, have adequate lighting, and be comfortable to use without requiring awkward positions. Keep the work space in the cell culture hood clean and uncluttered, and keep everything in direct line of sight. Disinfect each item placed in the cell culture hood by spraying them with 70% ethanol and wiping clean.

The arrangement of items within the cell culture hood usually adheres to the following right-handed convention, which can be modified to include additional items used in specific applications.

- A wide, clear work space in the center with your cell culture vessels
- Pipettor in the front right, where it can be reached easily
- Reagents and media in the rear right to allow easy pipetting
- Tube rack in the rear middle holding additional reagents
- Small container in the rear left to hold liquid waste

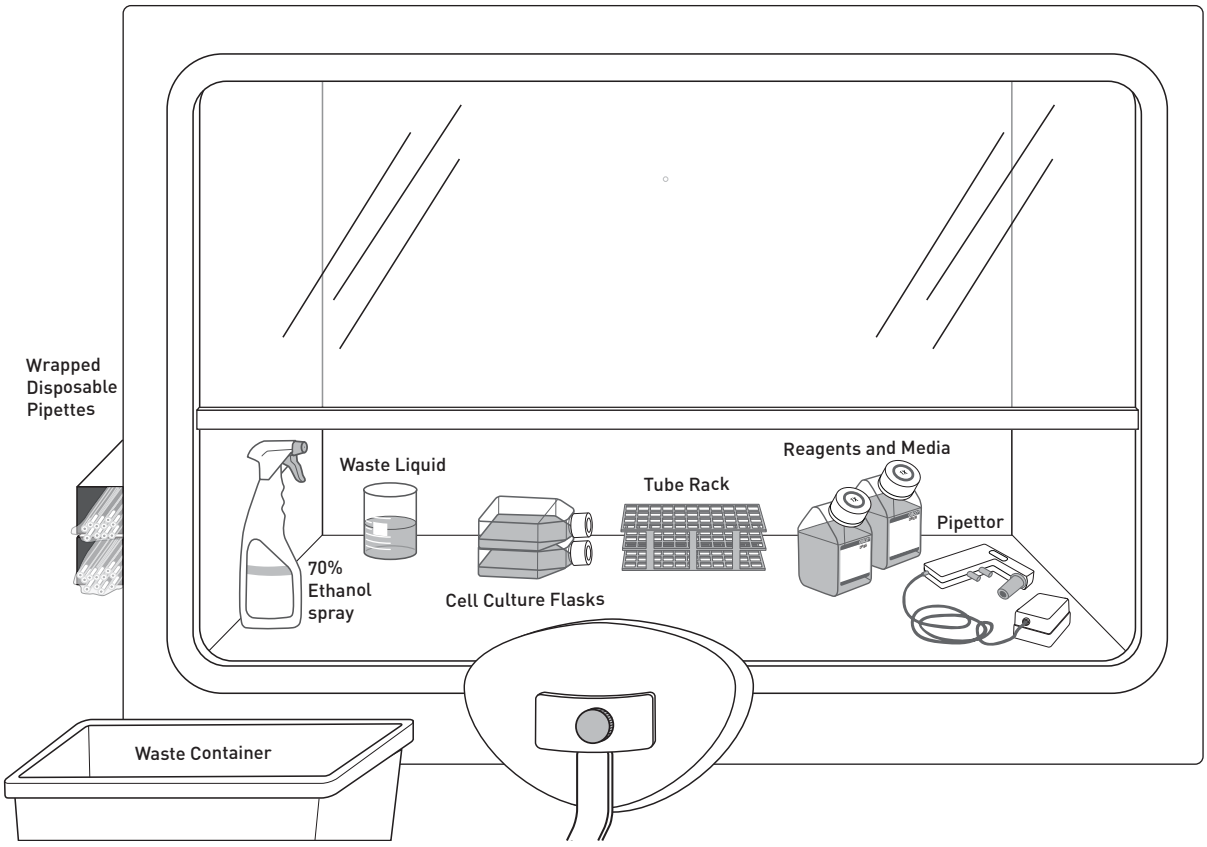


Figure 2.1. The basic layout of a cell culture hood for right-handed workers. Left-handed workers may switch the positions of the items laid out on the work surface.

Incubator

The purpose of the incubator is to provide the appropriate environment for cell growth. The incubator should be large enough for your laboratory needs, have forced-air circulation, and should have temperature control to within ±0.2°C. Stainless steel incubators allow easy cleaning and provide corrosion protection, especially if humid air is required for incubation. Although the requirement for aseptic conditions in a cell culture incubator is not as stringent as that in a cell culture hood, frequent cleaning of the incubator is essential to avoid contamination of cell cultures.

Types of Incubators

There are two basic types of incubators, dry incubators and humid CO_2 incubators. **Dry incubators** are more economical, but require the cell cultures to be incubated in sealed flasks to prevent evaporation. Placing a water dish in a dry incubator can provide some humidity, but they do not allow precise control of atmospheric conditions in the incubator. **Humid CO_2 incubators** are more expensive, but allow superior control of culture conditions. They can be used to incubate cells cultured in Petri dishes or multi-well plates, which require a controlled atmosphere of high humidity and increased CO_2 tension.

Storage

A cell culture laboratory should have storage areas for liquids such as media and reagents, for chemicals such as drugs and antibiotics, for consumables such as disposable pipettes, culture vessels, and gloves, for glassware such as media bottles and glass pipettes, for specialized equipment, and for tissues and cells.

Glassware, plastics, and specialized equipment can be stored at ambient temperature on shelves and in drawers; however, it is important to **store all media, reagents, and chemicals according to the instructions on the label**.

Some media, reagents, and chemicals are sensitive to light; while their normal laboratory use under lighted conditions is tolerated, they should be stored in the dark or wrapped in aluminum foil when not in use.

Refrigerators

For small cell culture laboratories, a domestic refrigerator (preferably one without a autodefrost freezer) is an adequate and inexpensive piece of equipment for storing reagents and media at 2–8°C. For larger laboratories, a cold room restricted to cell culture is more appropriate. Make sure that the refrigerator or the cold room is cleaned regularly to avoid contamination.

Freezers

Most cell culture reagents can be stored at –5°C to –20°C; therefore an ultradeep freezer (i.e., a –80°C freezer) is optional for storing most reagents. A domestic freezer is a cheaper alternative to a laboratory freezer. While most reagents can withstand temperature oscillations in an autodefrost (i.e., self-thawing) freezer, some reagents such as antibiotics and enzymes should be stored in a freezer that does not autodefrost.

Cryogenic Storage

Cell lines in continuous culture are likely to suffer from genetic instability as their passage number increases; therefore, it is essential to prepare working stocks of the cells and preserve them in cryogenic storage (for more information, see **Freezing Cells**, page 37). Do **not** store cells in –20°C or –80°C freezers, because their viability quickly decreases when they are stored at these temperatures.

There are two main types of liquid-nitrogen storage systems, vapor phase and liquid phase, which come as wide-necked or narrow-necked storage containers. **Vapor phase** systems minimize the risk of explosion with cryostorage tubes, and are required for storing biohazardous materials, while the **liquid phase** systems usually have longer static holding times, and are therefore more economical.

Narrow-necked containers have a slower nitrogen evaporation rate and are more economical, but **wide-necked** containers allow easier access and have a larger storage capacity.

Cell Counter

A cell counter is essential for quantitative growth kinetics, and a great advantage when more than two or three cell lines are cultured in the laboratory.

The Countess® Automated Cell Counter is a bench-top instrument designed to measure cell count and viability (live, dead, and total cells) accurately and precisely in less than a minute per sample, using the standard Trypan Blue uptake technique. Using the same amount of sample that you currently use with the hemacytometer, the Countess® Automated Cell Counter takes less than a minute per sample for a typical cell count and is compatible with a wide variety of eukaryotic cells.

Aseptic Technique

Introduction

Successful cell culture depends heavily on keeping the cells free from contamination by microorganisms such as bacterial, fungi, and viruses. Non-sterile supplies, media, and reagents, airborne particles laden with microorganisms, unclean incubators, and dirty work surfaces are all sources of biological contamination.

Aseptic technique, designed to provide a barrier between the microrganisms in the environment and the sterile cell culture, depends upon a set of procedures to reduce the probability of contamination from these sources. The elements of aseptic technique are a sterile work area, good personal hygiene, sterile reagents and media, and sterile handling.

Sterile Work Area

The simplest and most economical way to reduce contamination from airborne particles and aerosols (e.g., dust, spores, shed skin, sneezing) is to use a cell culture hood.

- The cell culture hood should be properly set up and be located in an area that is restricted to cell culture that is free from drafts from doors, windows, and other equipment, and with no through traffic.
- The work surface should be uncluttered and contain only items required for a particular procedure; it should not be used as a storage area.
- Before and after use, the work surface should be disinfected thoroughly, and the surrounding areas and equipment should be cleaned routinely.
- For routine cleaning, wipe the work surface with 70% ethanol before and during work, especially after any spillage.
- You may use ultraviolet light to sterilize the air and exposed work surfaces in the cell culture hood between uses.
- Using a Bunsen burner for flaming is not necessary nor recommended in a cell culture hood.
- Leave the cell culture hood running at all times, turning them off only when they will not be used for extended periods of time.

Good Personal Hygiene

Wash your hands before and after working with cell cultures. In addition to protecting you from hazardous materials, wearing personal protective equipment also reduces the probability of contamination from shed skin as well as dirt and dust from your clothes.

Sterile Reagents and Media

Commercial reagents and media undergo strict quality control to ensure their sterility, but they can become contaminated while handling. Follow the guidelines below for sterile handling to avoid contaminating them. Always sterilize any reagents, media, or solutions prepared in the laboratory using the appropriate sterilization procedure (e.g., autoclave, sterile filter).

Sterile Handling

- Always wipe your hands and your work area with 70% ethanol.
- Wipe the outside of the containers, flasks, plates, and dishes with 70% ethanol before placing them in the cell culture hood.
- Avoid pouring media and reagents directly from bottles or flasks.
- Use sterile glass or disposable plastic pipettes and a pipettor to work with liquids, and use each pipette only once to avoid cross contamination. Do not unwrap sterile pipettes until they are to be used. Keep your pipettes at your work area.
- Always cap the bottles and flasks after use and seal multi-well plates with tape or place them in resealable bags to prevent microorganisms and airborn contaminants from gaining entry.
- Never uncover a sterile flask, bottle, petri dish, etc. until the instant you are ready to use it and never leave it open to the environment. Return the cover as soon as you are finished.
- If you remove a cap or cover, and have to put it down on the work surface, place the cap with opening facing down.
- Use only sterile glassware and other equipment.
- Be careful not to talk, sing, or whistle when you are performing sterile procedures.
- Perform your experiments as rapidly as possible to minimize contamination.

Aseptic Technique Checklist

The following checklist provides a concise list of suggestions and procedures to guide you to achieve a solid aseptic technique. For an in-depth review of aseptic technique, refer to *Culture of Animal Cells: A Manual of Basic Technique* (Freshney, 2000).

Work Area	
Is the cell culture hood properly set up?	
Is the cell culture hood in an area free from drafts and through traffic?	
Is the work surface uncluttered, and does it contain only items required for your experiment?	
Did you wipe the work surface with 70% ethanol before work?	
Are you routinely cleaning and sterilizing your incubators, refrigerators, freezers, and other laboratory equipment?	
Personal Hygiene	
Did you wash your hands?	
Are you wearing personal protective equipment?	
If you have long hair, is it tied in the back?	
Are you using a pipettor to work with liquids?	
Reagents and Media	
Have you sterilized any reagents, media, and solutions you have prepared in the laboratory using the appropriate procedure?	
Did you wipe the outside of the bottles, flasks, and plates with 70% ethanol before placing them on your work surface?	
Are all your bottles, flasks, and other containers capped when not in use?	
Are all your plates stored in sterile resealable bags?	
Does any of your reagents look cloudy? Contaminated? Do they contain floating particles? Have foul smell? Unusual color? If yes, did you decontaminated and discarded them?	
Handling	
Are you working slowly and deliberately, mindful of aseptic technique?	
Did you wipe the surfaces of all the items including pipettor, bottles, and flasks with 70% ethanol before placing them in the cell culture hood?	
Are placing the caps or covers face down on the work area?	
Are you using sterile glass pipettes or sterile disposable plastic pipettes to manipulate all liquids?	
Are you using a sterile pipette only once to avoid cross contamination?	
Are you careful **not** to touch the pipette tip to anything non-sterile, including the outside edge of the bottle threads?	
Did you mop up any spillage immediately, and wiped the area with 70% ethanol?	

Biological Contamination

Introduction

Contamination of cell cultures is easily the most common problem encountered in cell culture laboratories, sometimes with very serious consequences. Cell culture contaminants can be divided into two main categories, **chemical contaminants** such as impurities in media, sera, and water, endotoxins, plasticizers, and detergents, and **biological contaminants** such as bacteria, molds, yeasts, viruses, mycoplasma, as well as cross contamination by other cell lines. While it is impossible to eliminate contamination entirely, it is possible to reduce its frequency and seriousness by gaining a thorough understanding of their sources and by following good aseptic technique. This section provides an overview of major types of biological contamination.

Bacteria

Bacteria are a large and ubiquitous group of unicellular microorganisms. They are typically a few micrometers in diameters, and can have a variety of shapes, ranging from spheres to rods and spirals. Because of their ubiquity, size, and fast growth rates, bacteria, along with yeasts and molds, are the most commonly encountered biological contaminants in cell culture. Bacterial contamination is easily detected by visual inspection of the culture within a few days of it becoming infected; infected cultures usually appear cloudy (i.e., turbid), sometimes with a thin film on the surface. Sudden drops in the pH of the culture medium is also frequently encountered. Under a low-power microscope, the bacteria appear as tiny, moving granules between the cells, and observation under a high-power microscope can resolve the shapes of individual bacteria. The simulated images below show an adherent 293 cell culture contaminated with *E. coli*.

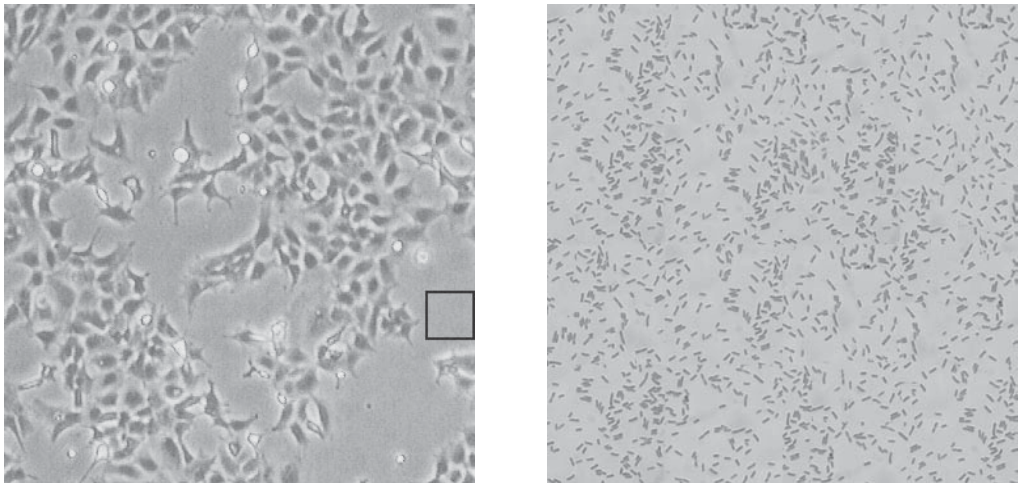


Figure 2.2. Simulated phase contrast images of adherent 293 cells contaminated with *E. coli*. The spaces between the adherent cells show tiny, shimmering granules under low power microscopy, but the individual bacteria are not easily distinguishable (panel A). Further magnification of the area enclosed by the black square resolves the individual *E. coli* cells, which are typically rod-shaped and are about 2 µm long and 0.5 µm in diameter. Each side of the black square in panel A is 100 µm.

Yeasts

Yeasts are unicellular eukaryotic microorganisms in the kingdom of Fungi, ranging in size from a few micrometers (typically) up to 40 micrometers (rarely). Like bacterial contamination, cultures contaminated with yeasts become turbid, especially if the contamination is in an advanced stage. There is very little change in the pH of the culture contaminated by yeasts until the contamination becomes heavy, at which stage the pH usually increases. Under microscopy, yeast appear as individual ovoid or spherical particles, that may bud off smaller particles. The simulated image below shows adherent 293 cell culture 24 hours after plating that is infected with yeast.

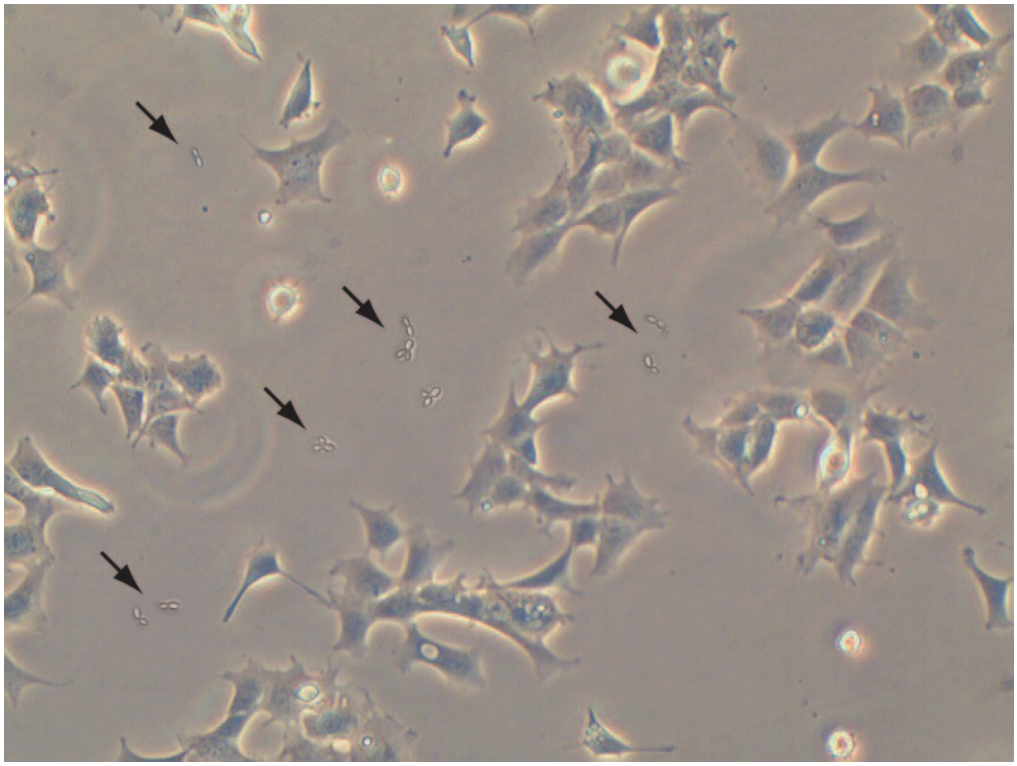


Figure 2.3. Simulated phase contrast images of 293 cells in adherent culture that is contaminated with yeast. The contaminating yeast cells appear as ovoid particles, budding off smaller particles as they replicate.

Molds

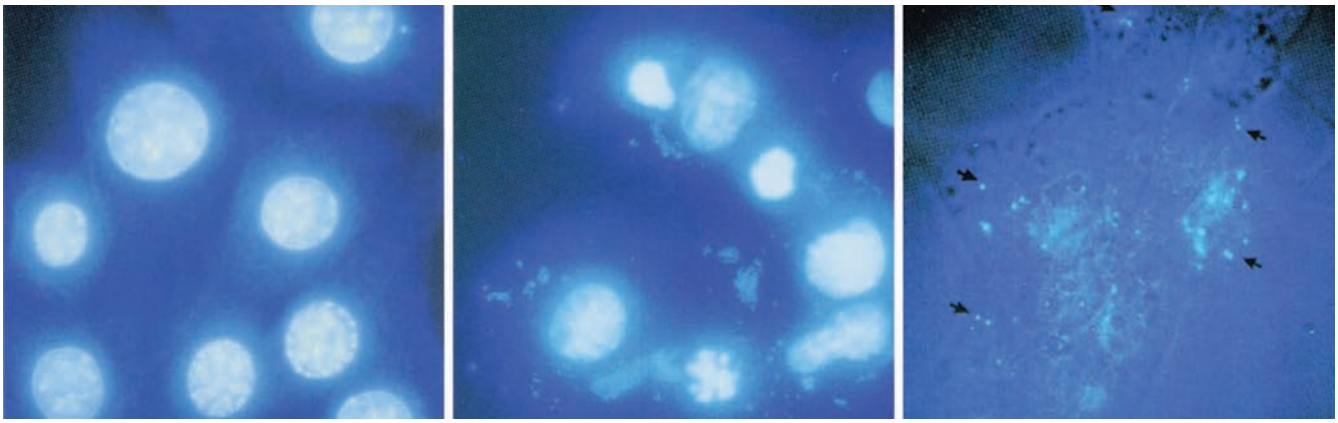
Molds are eukaryotic microorganisms in the kingdom of Fungi that grow as multicellular filaments called hyphae. A connected network of these multicellular filaments contain genetically identical nuclei, and are referred to as a colony or mycelium. Similar to yeast contamination, the pH of the culture remains stable in the initial stages of contamination, then rapidly increases as the culture become more heavily infected and becomes turbid. Under microscopy, the mycelia usually appear as thin, wisp-like filaments, and sometimes as denser clumps of spores. Spores of many mold species can survive extremely harsh and inhospitable environments in their dormant stage, only to become activated when they encounter suitable growth conditions.

Viruses

Viruses are microscopic infectious agents that take over the host cells machinery to reproduce. Their extremely small size makes them very difficult to detect in culture, and to remove them from reagents used in cell culture laboratories. Because most viruses have very stringent requirements for their host, they usually do not adversely effect cell cultures from species other than their host. However, using virally infected cell cultures can present a serious health hazard to the laboratory personnel, especially if human or primate cells are cultured in the laboratory. Viral infection of cell cultures can be detected by electron microscopy, immunostaining with a panel of antibodies, ELISA assays, or PCR with appropriate viral primers.

Mycoplasma

Mycoplasma are simple bacteria that lack a cell wall, and they are considered the smallest self-replicating organism. Because of their extremely small size (typically less than one micrometer), mycoplasma are very difficult to detect until they achieve extremely high densities and cause the cell culture to deteriorate; until then, there are often no visible signs of infection. Some slow growing mycoplasma may persists in culture without causing cell death, but they can alter the behavior and metabolism of the host cells in the culture. Chronic mycoplasma infections might manifest themselves with decreased rate of cell proliferation, reduced saturation density, and agglutination in suspension cultures; however, the only assured way of detecting mycoplasma contamination is by testing the cultures periodically using fluorescent staining (e.g., Hoechst 33258), ELISA, PCR, immunostaining, autoradiography, or microbiological assays.



A B C

Figure 2.4. Photomicrographs of mycoplasma-free cultured cells (panel A) and cells infected with mycoplasma (panels B and C). The cultures were tested using the MycoFluor™ Mycoplasma Detection Kit, following the kit protocols. In fixed cells, the MycoFluor™ reagent has access to the cell nuclei, which are intensely stained with the reagent, but the absence of fluorescent extranuclear objects indicates that the culture is free from mycoplasma contamination (panel A**)**. In fixed cells infected with mycoplasma, the MycoFluor™ reagent stains both the nuclei and the mycoplasma, but the intense relative fluorescence of the nuclei obscure the mycoplasma on or near the nuclei. However, the mycoplasma separated from the bright nuclei are readily visible (panel B). In live cells, the MycoFluor™ reagent does not have access to the nuclei, but readily stains the mycoplasma associated with the outside of cells (panel C). The images were obtained using 365 nm excitation and a 100/1.3 Plan Neoflaur® (Zeiss) objective lens coupled with a 450 ± 30 nm bandpass filter.

Cross-Contamination

While not as common as microbial contamination, extensive cross-contamination of many cell lines with HeLa and other fast growing cell lines is a clearly-established problem with serious consequences. Obtaining cell lines from reputable cell banks, periodically checking the characteristics of the cell lines, and practicing good aseptic technique are practices that will help you avoid cross-contamination. DNA fingerprinting, karyotype analysis, and isotype analysis can confirm the presence or absence of cross-contamination in your cell cultures.

Using Antibiotics

Antibiotics should not be used routinely in cell culture, because their continuous use encourages the development of antibiotic resistant strains and allows low-level contamination to persist, which can develop into full-scale contamination once the antibiotic is removed from media, and may hide mycoplasma infections and other cryptic contaminants. Further, some antibiotics might cross react with the cells and interfere with the cellular processes under investigation.

Antibiotics should only be used as a last resort and only for short term applications, and they should be removed from the culture as soon as possible. If they are used in the long term, antibiotic-free cultures should be maintained in parallel as a control for cryptic infections.

Cell Culture Basics

This section provides information on the fundamentals of cell culture, including the selection of the appropriate cell line for your experiments, media requirements for cell culture, adherent versus suspension culture, and morphologies of continuous cell lines available from Invitrogen.

Note that the following information is an introduction to the basics of cell culture, and it is intended as a starting point in your investigations. For more in-depth information, we recommend that you consult published literature and books, as well as the manuals and product information sheets provided with the products you are using.

Cell Lines

Selecting the Appropriate Cell Line

Consider the following criteria for selecting the appropriate cell line for your experiments:

- **Species:** Non-human and non-primate cell lines usually have fewer biosafety restrictions, but ultimately your experiments will dictate whether to use species-specific cultures or not.
- **Functional characteristics:** What is the purpose of your experiments? For example, liver- and kidney-derived cell lines may be more suitable for toxicity testing.
- **Finite or continuous:** While choosing from finite cell lines may give you more options to express the correct functions, continuous cell lines are often easier to clone and maintain.
- **Normal or transformed:** Transformed cell lines usually have an increased growth rate and higher plating efficiency, are continuous, and require less serum in media, but they have undergone a permanent change in their phenotype through a genetic transformation.
- **Growth conditions and characteristics:** What are your requirements with respect to growth rate, saturation density, cloning efficiency, and the ability to grow in suspension? For example, to express a recombinant protein in high yields, you might want to choose a cell line with a fast growth rate and an ability to grow in suspension.
- **Other criteria:** If you are using a finite cell line, are there sufficient stocks available? Is the cell line well-characterized, or do you have to perform the validation yourself? If you are using an abnormal cell line, do you have an equivalent normal cell line that you can use as a control? Is the cell line stable? If not, how easy it is to clone it and generate sufficient frozen stocks for your experiments?

Acquiring Cell Lines

You may establish your own culture from primary cells, or you may choose to buy established cell cultures from commercial or non-profit suppliers (i.e., cell banks). Reputable suppliers provide high quality cell lines that are carefully tested for their integrity and to ensure that the culture is free from contaminants. We advise against borrowing cultures from other laboratories because they carry a high risk of contamination. Regardless of their source, make sure that all new cell lines are tested for mycoplasma contamination before you begin to use them.

Invitrogen offers a variety of primary cultures and established cell lines, reagents, media, sera, and growth factors for your cell culture experiments. The **Appendix** section contains a list of the more commonly used cell lines available from Invitrogen (see page page 44). For more information on Invitrogen and GIBCO® products, refer to www.invitrogen.com.

Culture Environment

One of the major advantages of cell culture is the ability to manipulate the **physico-chemical** (i.e., temperature, pH, osmotic pressure, O_2 and CO_2 tension) and the **physiological environment** (i.e., hormone and nutrient concentrations) in which the cells propagate. With the exception of temperature, the culture environment is controlled by the growth media.

While the physiological environment of the culture is not as well defined as its physico-chemical environment, a better understanding of the components of serum, the identification of the growth factors necessary for proliferation, and a better appreciation of the microenvironment of cells in culture (i.e., cell-cell interactions, diffusion of gases, interactions with the matrix) now allow the culture of certain cell lines in serum-free media.

Adherent vs Suspension Culture

There are two basic systems for growing cells in culture, as monolayers on an artificial substrate (i.e., **adherent culture**) or free-floating in the culture medium (**suspension culture**). The majority of the cells derived from vertebrates, with the exception of hematopoietic cell lines and a few others, are anchorage-dependent and have to be cultured on a suitable substrate that is specifically treated to allow cell adhesion and spreading (i.e., **tissue-culture treated**). However, many cell lines can also be adapted for suspension culture. Similarly, most of the commercially available insect cell lines grow well in monolayer or suspension culture. Cells that are cultured in suspension can be maintained in culture flasks that are not tissue-culture treated, but as the culture volume to surface area is increased beyond which adequate gas exchange is hindered (usually 0.2–0.5 mL/cm^2), the medium requires agitation. This agitation is usually achieved with a magnetic stirrer or rotating spinner flasks.

Adherent Culture	Suspension Culture
Appropriate for most cell types, including primary cultures.	Appropriate for cells adapted to suspension culture and a few other cell lines that are nonadhesive (e.g., hematopoietic).
Requires periodic passaging, but allows easy visual inspection under inverted microscope.	Easier to passage, but requires daily cell counts and viability determination to follow growth patterns; culture can be diluted to stimulate growth.
Cells are dissociated enzymatically (e.g., TrypLE™ Express, trypsin) or mechanically.	Does not require enzymatic or mechanical dissociation.
Growth is limited by surface area, which may limit product yields.	Growth is limited by concentration of cells in the medium, which allows easy scale-up.
Requires tissue-culture treated vessel.	Can be maintained in culture vessels that are not tissue-culture treated, but requires agitation (i.e., shaking or stirring) for adequate gas exchange.
Used for cytology, harvesting products continuously, and many research applications.	Used for bulk protein production, batch harvesting, and many research applications.

Media

The culture medium is the most important component of the culture environment, because it provides the necessary nutrients, growth factors, and hormones for cell growth, as well as regulating the pH and the osmotic pressure of the culture.

Although initial cell culture experiments were performed using natural media obtained from tissue extracts and body fluids, the need for standardization, media quality, and increased demand led to the development of defined media. The three basic classes of media are **basal media**, **reduced-serum media**, and **serum-free media**, which differ in their requirement for supplementation with **serum**.

Serum is vitally important as a source of growth and adhesion factors, hormones, lipids and minerals for the culture of cells in basal media. In addition, serum also regulates cell membrane permeability and serves as a carrier for lipids, enzymes, micronutrients, and trace elements into the cell. However, using serum in media has a number of disadvantages including high cost, problems with standardization, specificity, variability, and unwanted effects such as stimulation or inhibition of growth and/or cellular function on certain cell cultures. If the serum is not obtained from reputable source, contamination can also pose a serious threat to successful cell culture experiments. To address this threat, all Invitrogen and GIBCO® products, including sera, are tested for contamination and guaranteed for their quality, safety, consistency, and regulatory compliance.

Basal Media

The majority of cell lines grow well in basal media, which contain amino acids, vitamins, inorganic salts, and a carbon source such as glucose, but these basal media formulations must be further supplemented with serum.

Reduced-Serum Media

Another strategy to reduce the undesired effects of serum in cell culture experiments is to use reduced-serum media. Reduced-serum media are basal media formulations enriched with nutrients and animal-derived factors, which reduce the amount of serum that is needed.

Serum-Free Media

Serum-free media (SFM) circumvents issues with using animal sera by replacing the serum with appropriate nutritional and hormonal formulations. Serum-free media formulations exist for many primary cultures and cell lines, including recombinant protein producing lines of Chinese Hamster Ovary (CHO), various hybridoma cell lines, the insect lines Sf9 and Sf21 (*Spodoptera frugiperda*), and for cell lines that act as hosts for viral production (e.g., 293, VERO, MDCK, MDBK), and others. One of the major advantages of using serum-free media is the ability to make the medium selective for specific cell types by choosing the appropriate combination of growth factors. The table below lists the advantages and disadvantages of serum-free media.

Advantages	Disadvantages
• Increased definition • More consistent performance • Easier purification and downstream processing • Precise evaluation of cellular functions • Increased productivity • Better control over physiological response • Enhanced detection of cellular mediators	• Requirement for cell type-specific media formulations • Need for higher degree of reagent purity • Slower growth

Invitrogen offers a wide range of classical basal media, reduced-serum media, and serum-free media, as well as sera, growth factors, supplements, antibiotics, and reagents for your cell culture experiments. The **Appendix** section contains a list of the more commonly used cell culture products available from Invitrogen. For more information on Invitrogen and GIBCO® cell culture products, refer to www.invitrogen.com.

pH

Most normal **mammalian cell lines** grow well at pH 7.4, and there is very little variability among different cell strains. However, some transformed cell lines have been shown to grow better at slightly more acidic environments (pH 7.0–7.4), and some normal fibroblast cell lines prefer slightly more basic environments (pH 7.4–7.7). **Insect cell lines** such as Sf9 and Sf21 grow optimally at pH 6.2.

CO_2

The growth medium controls the pH of the culture and buffers the cells in culture against changes in the pH. Usually, this buffering is achieved by including an organic (e.g., HEPES) or CO_2-bicarbonate based buffer. Because the pH of the medium is dependent on the delicate balance of dissolved carbon dioxide (CO_2) and bicarbonate (HCO_3^-), changes in the atmospheric CO_2 can alter the pH of the medium. Therefore, it is necessary to use exogenous CO_2 when using media buffered with a CO_2-bicarbonate based buffer, especially if the cells are cultured in open dishes or transformed cell lines are cultured at high concentrations. While most researchers usually use 5–7% CO_2 in air, 4–10% CO_2 is common for most cell culture experiments. However, each medium has a recommended CO_2 tension and bicarbonate concentration to achieve the correct pH and osmolality; refer to the media manufacturer's instructions for more information.

Temperature

The optimal temperature for cell culture largely depends on the body temperature of the host from which the cells were isolated, and to a lesser degree on the anatomical variation in temperature (e.g., temperature of the skin may be lower than the temperature of skeletal muscle). Overheating is a more serious problem than underheating for cell cultures; therefore, often the temperature in the incubator is set slightly lower than the optimal temperature.

- Most **human and mammalian cell lines** are maintained at 36°C to 37°C for optimal growth.
- **Insect cells** are cultured at 27°C for optimal growth; they grow more slowly at lower temperatures and at temperatures between 27°C and 30°C. Above 30°C, the viability of insect cells decreases, and the cells do not recover even after they are returned to 27°C.
- **Avian cell lines** require 38.5°C for maximum growth. Although these cells can also be maintained at 37°C, they will grow more slowly.
- Cell lines derived from **cold-blooded animals** (e.g., amphibians, cold-water fish) tolerate a wide temperature range between 15°C and 26°C.

Note that cell culture conditions vary for each cell type. The consequences of deviating from the culture conditions required for a particular cell type can range from the expression of aberrant phenotypes to a complete failure of the cell culture. We therefore recommend that you familiarize yourself with your cell line of interest, and closely follow the instructions provided with each product you are using in your experiments.

Cell Morphology

Regularly examining the **morphology** of the cells in culture (i.e., their shape and appearance) is essential for successful cell culture experiments. In addition to confirming the healthy status of your cells, inspecting the cells by eye and a microscope each time they are handled will allow you to detect any signs of contamination early on and to contain it before it spreads to other cultures around the laboratory.

Signs of deterioration of cells include granularity around the nucleus, detachment of the cells from the substrate, and cytoplasmic vacuolation. Signs of deterioration may be caused by a variety of reasons, including contamination of the culture, senescence of the cell line, or the presence of toxic substances in the medium, or they may simply imply that the culture needs a medium change. Allowing the deterioration to progress too far will make it irreversible.

Mammalian Cells

Variations in Mammalian Cell Morphology

Most mammalian cells in culture can be divided in to three basic categories based on their morphology.

- **Fibroblastic** (or fibroblast-like) cells are bipolar or multipolar and have elongated shapes. They grow attached to a substrate.
- **Epithelial-like** cells are polygonal in shape with more regular dimensions, and grow attached to a substrate in discrete patches.
- **Lymphoblast-like** cells are spherical in shape and they are usually grown in suspension without attaching to a surface.

In addition to the basic categories listed above, certain cells display morphological characteristics specific to their specialized role in host.

- **Neuronal cells** exist in different shapes and sizes, but they can roughly be divided into two basic morphological categories, **type I** with long axons used to move signals over long distances and **type II** without axons. A typical neuron projects cellular extensions with many branches from the cell body, which is referred to as a dendritic tree. Neuronal cells can be unipolar or pseudounipolar with the dendrite and axon emerging from same process, bipolar with the axon and single dendrite on opposite ends of the soma (the central part of the cell containing the nucleus), or multipolar with more than two dendrites.

Morphology of 293 Cells

The 293 cell line is a permanent line established from primary embryonic human kidney, which was transformed with sheared human adenovirus type 5 DNA. The adenoviral genes expressed in this cell line allow the cells to produce very high levels of recombinant proteins. Invitrogen offers several variants of the 293 cell line, including those adapted for high-density suspension culture in serum-free media. For more information, visit our mammalian cell culture pages on our website.

The phase contrast images below show the morphology of healthy 293 cells in adherent culture at 80% confluency (Figure 3.1) and in suspension culture (Figure 3.2). Note that adherent mammalian cultures should be passaged when they are in the log phase, before they reach confluence (see **When to Subculture** in the **Methods** chapter, page 27).

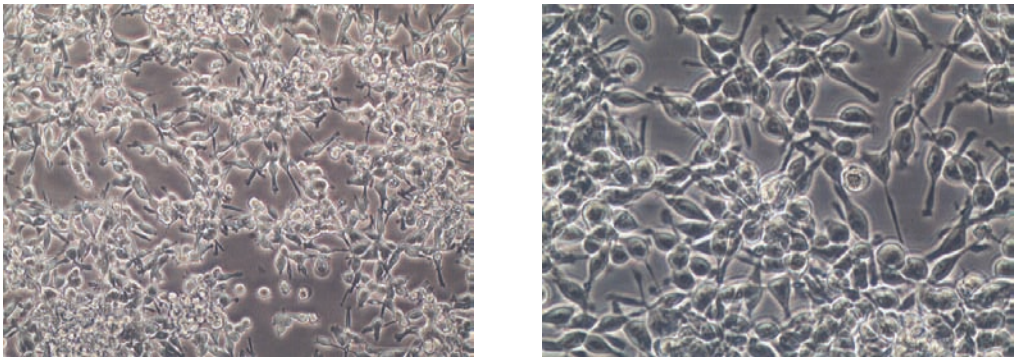


Figure 3.1. Phase contrast images of healthy 293 cells in adherent culture. The cells were plated at a seeding density of 5×10^4 viable cells/cm^2 in 293 SFM II medium and grown as a monolayer in a 37°C incubator with a humidified atmosphere of 5% CO_2 in air. The images were obtained using 10X and 20X objectives (panels A and B, respectively) 4 days after plating.

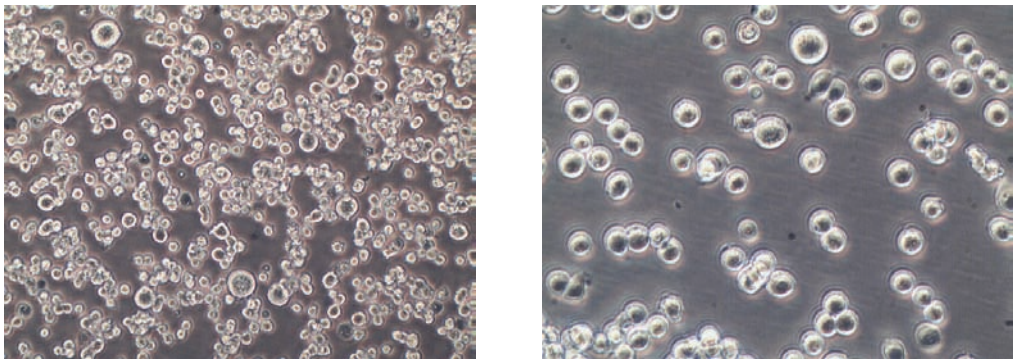


Figure 3.2. Phase contrast images of healthy 293F cells grown is suspension. The culture was started in a shake flask at a seeding density of 2×10^5 viable cells/mL in 293 SFM II medium and grown in a 37°C incubator with a humidified atmosphere of 5% CO_2 in air. 4 days after seeding, the cells were diluted 1:3, and the images were obtained using 10X and 20X objectives (panels A and B, respectively).

Insect Cells

Morphology of Sf21 Cells

Sf21 cells (IPLB-Sf21-AE) are ovarian cells isolated from *Spodoptera frugiperda* (Fall Armyworm). They are spherical in shape with unequal sizes, and have a somewhat granular appearance. Sf21 cells can be thawed and used directly in suspension culture for rapid expansion of cell stocks, propagation of baculovirus stocks, and production of recombinant proteins. Because Sf21 cells attach firmly to surfaces, they can be used as a monolayer for transfection or plaque assay applications.

The images below show the morphology of healthy Sf21 insect cells in suspension culture (Figure 3.3) and in adherent culture at confluency (Figure 3.4). Note that insect cells should be subcultured when they reach confluency (see **When to Subculture** in the **Methods** chapter, page 27).

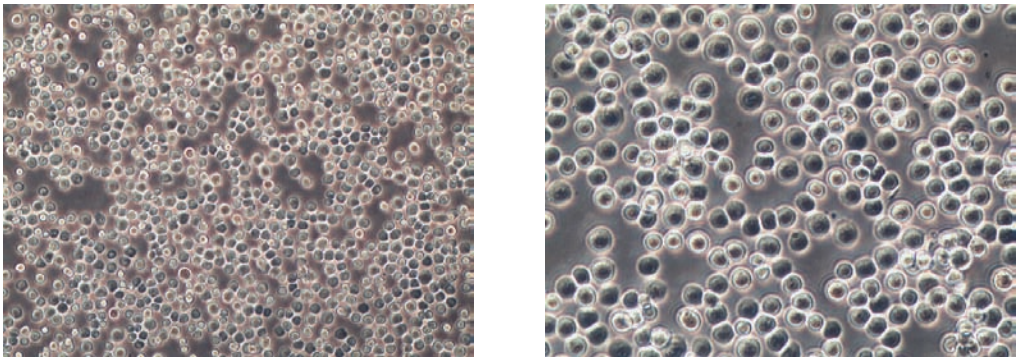


Figure 3.3. Phase contrast images of healthy Sf21 insect cells grown in suspension. The culture was started in a shake flask at a seeding density of 3×10^5 viable cells/mL in Sf-900 II SFM medium and it was maintained in a 28°C, non-humidified, ambient air-regulated incubator. The images were obtained using 10X and 20X objectives (panels A and B, respectively) 3 days after seeding.

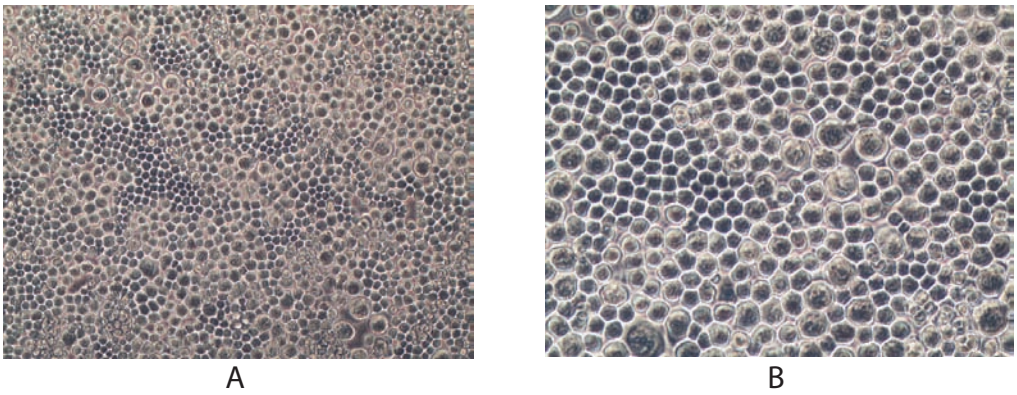


Figure 3.4. Phase contrast images of Sf21 insect cells grown as an adherent monolayer in 293 SFM II medium. The cells were plated at a seeding density of 5×10^4 viable cells/cm^2 in a T-25 flask and grown as monolayers in a 28°C, non-humidified, ambient air-regulated incubator. The images were obtained using 10X and 20X objectives (panels A and B, respectively) 7 days after seeding, when the culture had reached confluency.

Morphology of Sf9 Cells

The Sf9 insect cell line is a clonal isolate derived from the parental *Spodoptera frugiperda* cell line IPLB-Sf-21-AE, and it is a suitable host for expression of recombinant proteins from baculovirus expression systems (e.g., Invitrogen's Bac-to-Bac® and Bac-N-Blue™ Expression Systems). Although insect cells have been historically cultured in stationary systems utilizing T-flasks and serum-supplemented basal medium, insect cells are generally not anchorage dependent and can easily be maintained in suspension culture.

The images below show the morphology of healthy Sf9 insect cells in suspension and adherent cultures. Sf9 cells attach firmly to surfaces, and their small, regular size makes them exceptional for the formation of monolayers and plaques.

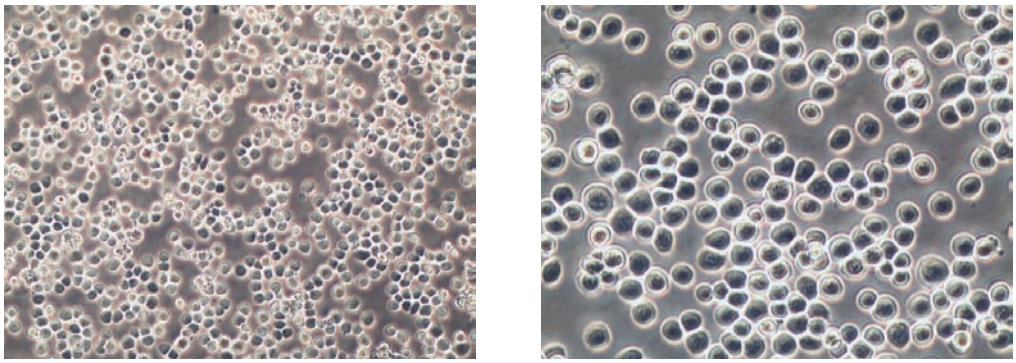


Figure 3.5. Phase contrast images of healthy Sf9 insect cells grown in suspension. The culture was started in a shake flask at a seeding density of 3×10^5 viable cells/mL in Sf-900 II SFM medium and it was maintained in a 28°C, non-humidified, ambient air-regulated incubator. The images were obtained using 10X and 20X objectives (panels A and B, respectively) 3 days after seeding.

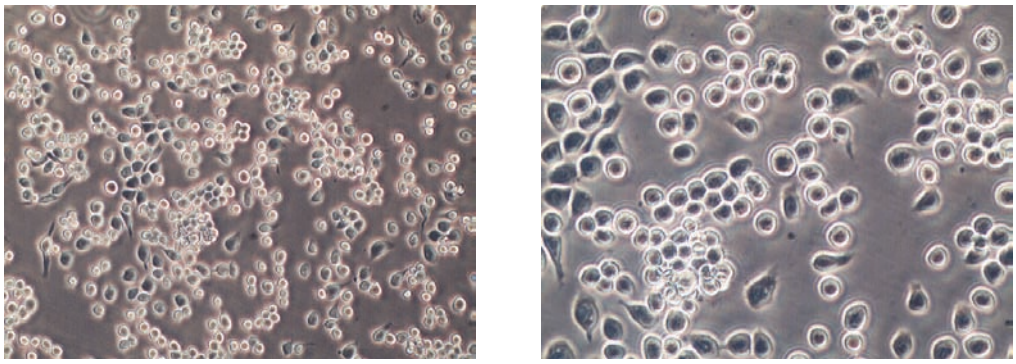


Figure 3.6. Phase contrast images of healthy Sf9 insect cells grown as an adherent monolayer in 293 SFM II medium. The cells were plated at a seeding density of 5×10^4 viable cells/cm^2 in a T-25 flask and grown as monolayers in a 28°C, non-humidified, ambient air-regulated incubator. The images were obtained using 10X and 20X objectives (panels A and B, respectively) 3 days after seeding.

Methods

Guidelines for Maintaining Cultured Cells

This section provides guidelines and general procedures for routine subculturing, thawing, and freezing of cells in culture. Note that cell culture conditions vary for each cell type. The consequences of deviating from the culture conditions required for a particular cell type can range from the expression of aberrant phenotypes to a complete failure of the cell culture. We therefore recommend that you familiarize yourself with your cell line of interest, and closely follow the instructions provided with each product you are using in your experiments.

What is Subculture?

Subculturing, also referred to as **passaging**, is the removal of the medium and transfer of cells from a previous culture into fresh growth medium, a procedure that enables the further propagation of the cell line or cell strain.

The growth of cells in culture proceeds from the **lag phase** following seeding to the **log phase**, where the cells proliferate exponentially. When the cells in adherent cultures occupy all the available substrate and have no room left for expansion, or when the cells in suspension cultures exceed the capacity of the medium to support further growth, cell proliferation is greatly reduced or ceases entirely (see Figure 4.1 below). To keep them at an optimal density for continued growth and to stimulate further proliferation, the culture has to divided and fresh medium supplied.

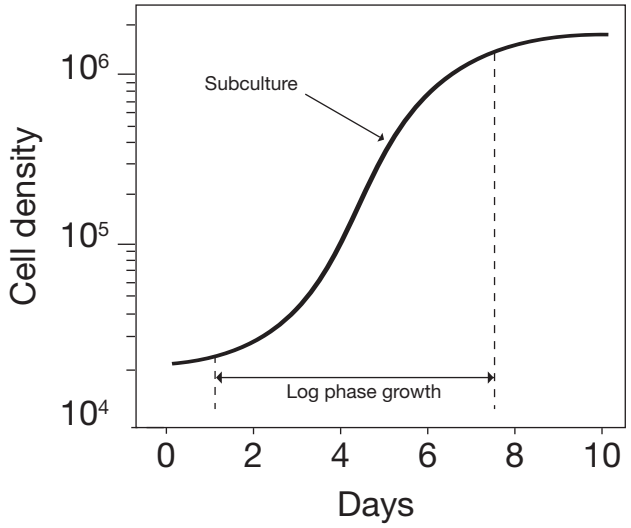


Figure 4.1. Characteristic Growth Pattern of Cultured Cells. The semi-logarithmic plot shows the cell density versus the time spent in culture. Cells in culture usually proliferate following a standard growth pattern. The first phase of growth after the culture is seeded is the **lag phase**, which is a period of slow growth when the cells are adapting to the culture environment and preparing for fast growth. The lag phase is followed by the **log phase** (i.e., "logarithmic" phase), a period where the cells proliferate exponentially and consume the nutrients in the growth medium. When all the growth medium is spent (i.e., one or more of the nutrients is depleted) or when the cells occupy all of the available substrate, the cells enter the **stationary phase** (i.e., plateau phase), where the proliferation is greatly reduced or ceases entirely.

When to Subculture?

The criteria for determining the need for subculture are similar in adherent and suspension cultures; however, there are some differences between mammalian and insect cell lines.

Cell Density

- **Mammalian cells:** Adherent cultures should be passaged when they are in the log phase, before they reach confluence. Normal cells stop growing when they reach confluence (**contact inhibition**), and it takes them longer to recover when reseeded. Transformed cells can continue proliferating even after they reach confluence, but they usually deteriorate after about two doublings. Similarly, cells in suspension should be passaged when they are in log-phase growth before they reach confluency. When they reach confluency, cells in suspension clump together and the medium appears turbid when the culture flask is swirled.
- **Insect cells:** Insect cells should be subcultured when they are in the log phase, before they reach confluency. While tightly adherent insect cells can be passaged at confluency, which allows for easier detachment from the culture vessel, insect cells that are repeatedly passaged at densities past confluency display decreased doubling times, decreased viabilities, and a decreased ability to attach. On the other hand, passaging insect cells in adherent culture before they reach confluency requires more mechanical force to dislodge them from the monolayer. When repeatedly subcultured before confluency, these cells also display decreased doubling times and decreased viabilities, and are considered unhealthy.

Exhaustion of Medium

- **Mammalian cells:** A drop in the pH of the growth medium usually indicates a build up of lactic acid, which is a by-product of cellular metabolism. Lactic acid can be toxic to the cells, and the decreased pH can be sub-optimal for cell growth. The rate of change of pH is generally dependent on the cell concentration in that cultures at a high cell concentration exhaust medium faster than cells lower concentrations. You should subculture your cells if you observe a rapid drop in pH (> 0.1–0.2 pH units) with an increase in cell concentration.
- **Insect cells:** Insect cells are cultured in growth media that are usually more acidic that those used for mammalian cells. For example, TNM-FH and Grace's medium used for culturing Sf9 cells has a pH of 6.2. Unlike mammalian cell cultures, the pH rises gradually as the insect cells grow, but usually does not exceed pH 6.4. However, as with mammalian cells, the pH of the growth medium will start falling when insect cells reach higher densities.

Subculture Schedule

Passaging your cells according to a strict schedule ensures reproducible behavior and allows you to monitor their health status. Vary the seeding density of your cultures until you achieve consistent growth rate and yield appropriate for your cell type from a given seeding density. Deviations from the growth patterns thus established usually indicate that the culture is unhealthy (e.g., deterioration, contamination) or a component of your culture system is not functioning properly (e.g., temperature is not optimal, culture medium too old). We strongly recommend that you keep a detailed **cell culture log**, listing the feeding and subculture schedules, types of media used, the dissociation procedure followed, split ratios, morphological observations, seeding concentrations, yields, and any anti-biotic use.

It is best to perform experiments and other non-routine procedures (e.g., changing type of media) according to your subculture schedule. If your experimental schedule does not fit the routine subculture schedule, make sure that you do not passage your cells while they are still in the lag period or when they have reached confluency and ceased growing.

Media Recommendations

Many continuous mammalian cell lines can be maintained on a relatively simple medium such as MEM supplemented with serum, and a culture grown in MEM can probably be just as easily grown in DMEM or Medium 199. However, when a specialized function is expressed, a more complex medium may be required. Information for selecting the appropriate medium for a given cell type is usually available in published literature, and may also be obtained from the source of the cells or cell banks.

If there is no information available on the appropriate medium for your cell type, choose the growth medium and serum empirically or test several different media for best results. In general, a good place to start is MEM for adherent cells and RPMI-1640 for suspension cells. The conditions listed below can be used as a guide line when setting up a new mammalian cell culture.

Insect cells are cultured in growth media that are usually more acidic that those used for mammalian cells such as TNM-FH and Grace's medium

Mammalian Cell Culture

Cell Line	Cell Type	Species	Tissue	Medium*
293	fibroblast	human	embryonic kidney	MEM and 10% FBS
3T6	fibroblast	mouse	embryo	DMEM, 10% FBS
A549	epithelial	human	lung carcinoma	F-12K, 10% FBS
A9	fibroblast	mouse	connective tissue	DMEM, 10% FBS
AtT-20	epithelial	mouse	pituitary tumor	F-10, 15% horse serum, and 2.5% FBS
BALB/3T3	fibroblast	mouse	embryo	DMEM, 10% FBS
BHK-21	fibroblast	hamster	kidney	GMEM, 10% FBS, or MEM, 10% FBS, and NEAA
BHL-100	epithelial	human	breast	McCoy'5A, 10% FBS
BT	fibroblast	bovine	turbinate cells	MEM, 10% FBS, and NEAA
Caco-2	epithelial	human	colon adeno carcinoma	MEM, 20% FBS, and NEAA
Chang	epithelial	human	liver	BME, 10% calf serum
CHO-K1	epithelial	hamster	ovary	F-12, 10% FBS
Clone 9	epithelial	rat	liver	F-12K, 10% FBS
Clone M-3	epithelial	mouse	melanoma	F-10, 15% horse serum, and 2.5% FBS
COS-1, COS-3, COS-7	fibroblast	monkey	kidney	DMEM, 10% FBS
CRFK	epithelial	cat	kidney	MEM, 10% FBS, and NEAA
CV-1	fibroblast	monkey	kidney	MEM, 10% FBS
D-17	epithelial	dog	osteosarcoma	MEM, 10% FBS, and NEAA
Daudi	lymphoblast	human	blood from a lymphoma patient	RPMI-1640, 10% FBS
GH1, GH3	epithelial	rat	pituitary tumor	F-10, 15% horse serum, and 2.5% FBS

* **BME**: Basal Medium Eagle; **DMEM**: Dulbecco's Modified Eagle Medium; **FBS**: Fetal Bovine Serum; **GMEM**: Glasgow Minimum Essential Medium; **IMDM**: Iscove's Modified Dulbecco's Medium; **MEM**: Minimum Essential Medium; **NEAA**: Non-Essential Amino Acids Solution.

Mammalian Cell Culture, continued				
Cell Line	**Cell Type**	**Species**	**Tissue**	**Medium***
H9	lymphoblast	human	T-cell lymphoma	RPMI-1640, 20% FBS
HaK	epithelial	hamster	kidney	BME, 10% calf serum
HCT-15	epithelial	human	colorectal adenocarcinoma	RPMI-1640, 10% FBS
HeLa	epithelial	human	cervix carcinoma	MEM, 10% FBS, and NEAA (in suspension, S-MEM)
HEp-2	epithelial	human	larynx carcinoma	MEM, 10% FBS
HL-60	lymphoblast	human	promyeolocytic leukemia	RPMI-1640, 20% FBS
HT-1080	epithelial	human	fibrosarcoma	MEM, 10% HI FBS, and NEAA
HT-29	epithelial	human	colon adenocarcinoma	McCoy's 5A, 10% FBS
HUVEC	endothelial	human	umbilical cord	F-12K, 10% FBS, and 100 µg/mL heparin
I-10	epithelial	mouse	testicular tumor	F-10, 15% horse serum, and 2.5% FBS
IM-9	lymphoblast	human	marrow from myeloma patient	RPMI-1640, 10% FBS
JEG-2	epithelial	human	choriocarcinoma	MEM, 10% FBS
Jensen	fibroblast	rat	sarcoma	McCoy's 5A, 5% FBS
Jurkat	lymphoblast	human	lymphoma	RPMI-1640, 10% FBS
K-562	lymphoblast	human	myelogenous leukemia	RPMI-1640, 10% FBS
KB	epithelial	human	oral carcinoma	MEM, 10% FBS, and NEAA
KG-1	myeloblast	human	marrow from erythroleukemia patient	IMDM, 20% FBS
L2	epithelial	rat	lung	F-12K, 10%FBS
LLC-WRC 256	epithelial	rat	carcinoma	Medium 199, 5% horse serum
McCoy	fibroblast	mouse	unknown	MEM, 10% FBS
MCF7	epithelial	human	breast adenocarcinoma	MEM, 10% FBS, NEAA, and 10 µg/mL insulin
WI-38	epithelial	human	embryonic lung	BME, 10% FBS
WISH	epithelial	human	amnion	BME, 10% FBS
XC	epithelial	rat	sarcoma	MEM, 10% FBS, and NEAA
Y-1	epithelial	mouse	tumor of adrenal	F-10, 15% horse serum, and 2.5% FBS
Insect Cell Culture				
Sf9, Sf21	fall army worm (*Spodoptera frugiperda*)		pupal ovary	TNM-FH and 10% FBS, or Sf-900 II SFM (serum-free), or Sf-900™ III SFM (serum-free)
High Five™ (BTI-TN-5B1-4)	cabbage looper (*Trichoplusia ni*)		ovary	TNM-FH and 10% FBS, or Express Five® SFM (serum-free)
Schneider 2 (S2), D.Mel-2	fruit fly *(Drosophila melanogaster)*			Schneider's *Drosophila* medium and 10% heat-inactivated FBS

*** BME**: Basal Medium Eagle; **DMEM**: Dulbecco's Modified Eagle Medium; **FBS**: Fetal Bovine Serum; **GMEM**: Glasgow Minimum Essential Medium; **IMDM**: Iscove's Modified Dulbecco's Medium; **MEM**: Minimum Essential Medium; **NEAA**: Non-Essential Amino Acids Solution; **TNM-FH:** *Trichoplusia ni* Medium-Formulation Hink (i.e., Grace's Insect Medium, Supplemented).

Dissociating Adherent Cells

The first step in subculturing adherent cells is to detach them from the surface of the culture vessel by enzymatic or mechanical means. The table below lists the various cell dissociation procedures.

Procedure	Dissociation Agent	Applications
Shake-off	Gentle shaking or rocking of culture vessel, or vigorous pipetting.	Loosely adherent cells, mitotic cells
Scraping	Cell scraper	Cell lines sensitive to proteases; may damage some cells
Enzymatic dissociation	Trypsin	Strongly adherent cells
	Trypsin + collagenase	High density cultures, cultures that have formed multiple layers, especially fibroblasts
	Dispase	Detaching epidermal cells as confluent, intact sheets from the surface of culture dishes without dissociating the cells
	TrypLE™ dissociation enzyme	Strongly adherent cells; direct substitute for trypsin; applications that require animal origin-free reagents

TrypLE™ Dissociation Enzymes

TrypLE™ Express and TrypLE™ Select are microbially produced cell dissociation enzymes with similar kinetics and cleavage specificities to trypsin. Although TrypLE™ enzymes can directly substitute trypsin in dissociation procedures without a need for protocol changes, we recommend that you initially optimize the incubation time for dissociation for best results. Because TrypLE™ enzymes are recombinant fungal trypsin-like proteases, they are ideal for applications that require animal origin-free reagents. The table below compares TrypLE™ Express and TrypLE™ Select to trypsin.

TrypLE™ Express and TrypLE™ Select	Trypsin
Completely free of animal- and human-derived components	Porcine- or bovine-derived
Stable at room temperature for at least six months.	Not stable at room temperature.
Does not require inactivation	Requires inactivation with serum or other inhibitors
Does not require trypsin inactivators	Requires trypsin inactivators

Subculturing Adherent Cells

The following protocol describes a **general procedure for subculturing adherent mammalian cells in culture**. Note that the procedure for passaging insect cells differs from that for mammalian cells on several crucial steps. For more information, refer to **Notes on Subculturing Insect Cells**, next page.

For passaging your own cell line, we recommend that you closely follow the instructions provided with each product you are using in your experiments. The consequences of deviating from the culture conditions required for a particular cell type can range from the expression of aberrant phenotypes to a complete failure of the cell culture.

Materials Needed

- Culture vessels containing your adherent cells
- Tissue-culture treated flasks, plates or dishes
- Complete growth medium, pre-warmed to 37°C
- Disposable, sterile 15-mL tubes
- 37°C incubator with humidified atmosphere of 5% CO_2
- Balanced salt solution such as Dulbecco's Phosphate Buffered Saline (DPBS), containing no calcium, magnesium, or phenol red
- Dissociation reagent such as trypsin or TrypLE™ Express, without phenol red
- Reagents and equipment to determine viable and total cell counts such as Countess® Automated Cell Counter, Trypan Blue and hemacytometer, or Coulter Counter® (Beckman Coulter)

Protocol for Passaging Adherent Cells

All solutions and equipment that come in contact with the cells must be sterile. Always use proper sterile technique and work in a laminar flow hood.

1. Remove and discard the spent cell culture media from the culture vessel.
2. Wash cells using a balanced salt solution without calcium and magnesium (approximately 2 mL per 10 cm^2 culture surface area). Gently add wash solution to the side of the vessel opposite the attached cell layer to avoid disturbing the cell layer, and rock the vessel back and forth several times.

   Note: The wash step removes any traces of serum, calcium, and magnesium that would inhibit the action of the dissociation reagent.

3. Remove and discard the wash solution from the culture vessel
4. Add the pre-warmed dissociation reagent such as trypsin or TrypLE™ to the side of the flask; use enough reagent to cover the cell layer (approximately 0.5 mL per 10 cm^2). Gently rock the container to get complete coverage of the cell layer.
5. Incubate the culture vessel at room temperature for approximately 2 minutes. Note that the actual incubation time varies with the cell line used.
6. Observe the cells under the microscope for detachment. If cells are less than 90% detached, increase the incubation time a few more minutes, checking for dissociation every 30 seconds. You may also tap the vessel to expedite cell detachment.

7. When ≥ 90% of the cells have detached, tilt the vessel for a minimal length of time to allow the cells to drain. Add the equivalent of 2 volumes (twice the volume used for the dissociation reagent) of pre-warmed complete growth medium. Disperse the medium by pipetting over the cell layer surface several times.
8. Transfer the cells to a 15-mL conical tube and centrifuge then at 200 × g for 5 to 10 minutes. Note that the centrifuge speed and time vary based on the cell type.
9. Resuspend the cell pellet in a minimal volume of pre-warmed complete growth medium and remove a sample for counting.
10. Determine the total number of cells and percent viability using a hemacytometer, cell counter and Trypan Blue exclusion, or the Countess® Automated Cell Counter. If necessary, add growth media to the cells to achieve the desired cell concentration and recount the cells.

    Note: We recommend using the Countess® Automated Cell Counter to determine the total number of cells and percent viability. Using the same amount of sample that you currently use with the hemacytometer, the Countess® Automated Cell Counter takes less than a minute per sample for a typical cell count and is compatible with a wide variety of eukaryotic cells. For more information on using a traditional hemacytometer, see **Support Protocols**, page 41.

11. Dilute cell suspension to the seeding density recommended for the cell line, and pipet the appropriate volume into new cell culture vessels, and return the cells to the incubator.

    Note: If using culture flasks, loosen the caps before placing them in the incubator to allow proper gas exchange unless you are using vented flasks with gas-permeable caps.

Notes on Subculturing Adherent Insect Cells

While the general procedure for subculturing insect cells follows the same steps as mammalian cells, some key requirements of these culture systems are different. For best results, always follow the instructions provided with each product you are using in your experiments.

- Passage insect cells at log phase. However, if your insect cells are strongly adherent, you may passage them at confluency or slightly after when they are starting to pull away from the bottom of the flask because they will be easier to dislodge.
- Densities lower than 20% confluency inhibit growth. The healthiest cells are those taken from log phase cultures.
- CO_2 exchange is not recommended for insect cell culture.
- Maintain insect cells at 27°C in a non-humidified environment. Cells can be maintained at room temperature on the bench top if protected from light or in a drawer. However, a 27°C controlled environment is recommended.
- Use media specifically formulated for insect cell growth.
- Insect cells attach very tightly to substrates under serum-free conditions and require additional effort to detach. To dislodge the cells, you may need to give the flask **one** quick shake using a wrist-snapping motion. To avoid contamination, always tighten the cap before this procedure.

Caution: We do not recommend shaking the flask vigorously, because it may result in damage to the cells.

Subculturing Suspension Cells

The following protocols describe **general procedures for subculturing mammalian cells in suspension culture**. Note that the procedure for passaging insect cells differs from that for mammalian cells on several crucial steps. For more information, refer to **Notes on Subculturing Insect Cells**, page 36.

For passaging your own cell line, we recommend that you closely follow the instructions provided with each product you are using in your experiments. The consequences of deviating from the culture conditions required for a particular cell type can range from the expression of aberrant phenotypes to a complete failure of the cell culture.

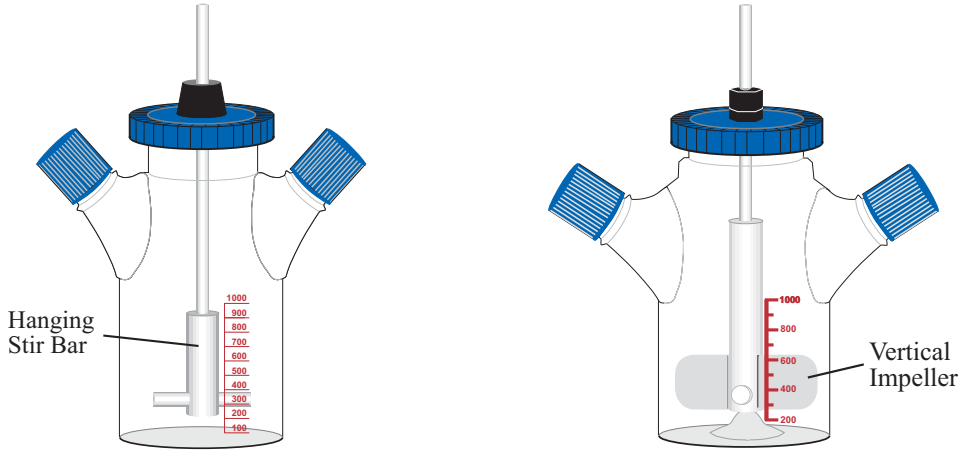
Passaging Suspension Cultures

Subculturing suspension cells is somewhat less complicated than passaging adherent cells. Because the cells are already suspended in growth medium, there is no need to treat them enzymatically to detach them from the surface of the culture vessel, and the whole process is faster and less traumatic for the cells. Replacement of growth medium is not carried out in suspension cultures; instead, the cells are maintained by feeding them every 2 to 3 days until they reach confluency. This can be done by directly diluting the cells in the culture flask and continue expanding them, or by withdrawing a portion of the cells from the culture flask and diluting the remaining cells down to a seeding density appropriate for the cell line. Usually, the lag period following the passaging is shorter than that observed with adherent cultures.

Suspension Culture Vessels

Suspension cultures can be maintained in sterile culture flasks (e.g., **shaker flasks** without baffles) that are not tissue-culture treated; however, **spinner flasks** (i.e., stirrer bottles) specifically designed for suspension cell culture allow for superior gas exchange and permit higher volumes of cells to be cultured.

Spinner flasks have two basic designs; the medium is agitated (i.e., stirred) by a hanging stir-bar assembly or with a vertical impeller. The vertical impeller provides better aeration. The total culture volume in a spinner flask should not exceed half of the indicated volume of the spinner for proper aeration (e.g., a 500 mL spinner should never contain more than 250 mL of culture).



Materials Needed

- Culture vessels containing your suspension cells
- Shaker flasks without baffles or spinner bottles (see **Suspension Culture Vessels**, previous page)
- Complete growth medium, pre-warmed to 37°C
- 37°C incubator with humidified atmosphere of 5% CO_2
- Magnetic stir plate (if using spinner flasks), roller rack (if using roller bottles), or shaking platform (if using conventional culture flasks or petri dishes)
- Reagents and equipment to determine viable and total cell counts (e.g., Countess® Automated Cell Counter, Trypan Blue and hemacytometer, or Coulter Counter®)

Protocol for Passaging Suspension Cells

All solutions and equipment that come in contact with the cells must be sterile. Always use proper sterile technique and work in a laminar flow hood. Subculture cells when they are in log-phase growth before they reach confluency. When they reach confluency, cells in suspension clump together and the medium appears turbid when the culture flask is swirled. The maximum recommended cell density before passaging varies with cell lines; refer to the cell-specific product insert or manual for details.

Cells Grown in Shaker Flasks

The following protocol describes a **general procedure** for passaging mammalian cells grown in suspension culture using shaker flasks in a shaking incubator. **For detailed protocols, always refer to the cell-specific product insert.**

Note: Make sure that the shaker flask does **not** have baffles (i.e., the indents at the bottom of the flask designed to provide agitation), because they ruin the shaking rhythm.

1. When the cells are ready for passaging (i.e., log-phase growth before they reach confluency), remove the flask from the incubator and take a small sample from the culture flask using a sterile pipette. If cells have settled down before taking the sample, swirl the flask to evenly distribute the cells in the medium.
2. From the sample, determine the total number of cells and percent viability using the Countess® Automated Cell Counter or a hemacytometer, cell counter, and Trypan Blue exclusion.
3. Calculate the volume of media that you need to add to dilute the culture down to the recommended seeding density.
4. Aseptically add the appropriate volume of pre-warmed growth medium into the culture flask. You may split the culture to multiple flasks if needed.
5. Loosen the caps of the culture flasks one full turn to allow for proper gas exchange (or use a gas-permeable cap), and return the flasks to the shaking incubator. The shaking speed depends on the cell line.

Note: To minimize the accumulation of cell debris and metabolic waste by-products in shaker cultures, gently centrifuge the cell suspension at 100 × *g* for 5 to 10 minutes, and resuspend the cell pellet in fresh growth medium once every three weeks (or as needed).

Cells Grown in Spinner Flasks

The following protocol describes a **general procedure** for passaging mammalian cells in suspension grown using spinner flasks. **For detailed protocols, always refer to the cell-specific product insert.**

Note that cells are sensitive to physical shearing. Ensure that impeller mechanisms rotate freely and do not contact vessel walls or the base. The top of the paddles should be slightly above the medium to ensure adequate aeration to the culture. Adjust the spinner mechanism so that paddles clear the sides and the bottom of the vessel. The table below lists the minimum volumes of media needed for different spinner flask sizes.

Size of Spinner Flask	Minimum Media Volume
100 mL	30 mL
250 mL	80 mL
500 mL	200 mL

We do not recommend initiating a spinner culture into a spinner flask larger than 500 mL. We suggest scaling up from smaller spinners that have already been established.

1. When the cells are ready for passaging (i.e., log-phase growth before they reach confluency), remove the flask from the incubator, and take a small sample from the culture flask using a sterile pipette. If cells have settled down before taking the sample, swirl the to evenly distribute the cells in the medium.
2. From the sample, determine the total number of cells and percent viability using the Countess® Automated Cell Counter or a hemacytometer, cell counter and Trypan Blue exclusion.
3. Calculate the volume of media that you need to add to dilute the culture down to the recommended seeding density.
4. Aseptically add the appropriate volume of pre-warmed growth medium into the culture flask. You may split the culture to multiple flasks if needed.
5. Loosen the side arm caps of the spinner flasks one full turn to allow for proper gas exchange, and return the flasks to the incubator. The spinner speed depends on the cell line and the impeller type. Make sure that the spinner speed is kept within the recommended values to avoid damage to the cells from shear stress.

Note: To minimize the accumulation of cell debris and metabolic waste by-products in spinner cultures, gently centrifuge the cell suspension at 100 × *g* for 5 to 10 minutes, and resuspend the cell pellet in fresh growth medium once every three weeks (or as needed).

Notes on Subculturing Suspension Insect Cells

While the general procedure for subculturing insect cells follows the same steps as mammalian cells, some key requirements of these culture systems are different. **For best results, always follow the instructions provided with the insect cell lines you are using in your experiments.**

- It is not necessary to change medium when you are culturing cells in suspension. Regular subculturing requires the removal of cell suspension and the addition of medium sufficient to dilute culture to the appropriate density (refer to the cell-specific product insert). Adding fresh medium is sufficient to replenish cell nutrients.
- CO_2 exchange is not recommended for insect cell culture.
- Maintain insect cells at 27°C in a non-humidified environment. Cells can be maintained at room temperature on the bench top or in a drawer, however, a 27°C controlled environment is recommended.
- Use media specifically formulated for insect cell growth.
- Use a surfactant to decrease shearing. 0.1% Pluronic® F-68 is recommended for spinner insect cultures. Pluronic® F-68 (BASF) is a surfactant that decreases cell membrane shearing due to impeller forces.
  Note: Sf-900 II SFM and Express Five® SFM already contain surfactants.
- Certain insect cell lines may require adaptation to suspension culture. For more information, refer to the cell-line specific product insert or manual.

Freezing Cells

Cryopreservation

Cell lines in continuous culture are prone to genetic drift, finite cell lines are fated for senescence, all cell cultures are susceptible to microbial contamination, and even the best-run laboratories can experience equipment failure. Because an established cell line is a valuable resource and its replacement is expensive and time consuming, it is vitally important that they are frozen down and preserved for long-term storage.

As soon as a small surplus of cells becomes available from subculturing, they should be frozen as a **seed stock**, protected, and not be made available for general laboratory use. **Working stocks** can be prepared and replenished from frozen seed stocks. If the seed stocks become depleted, cryopreserved working stocks can then serve as a source for preparing a fresh seed stock with a minimum increase in generation number from the initial freezing.

The best method for cryopreserving cultured cells is storing them in liquid nitrogen in complete medium in the presence of a cryoprotective agent such as dimethylsulfoxide (DMSO). Cryoprotective agents reduce the freezing point of the medium and also allow a slower cooling rate, greatly reducing the risk of ice crystal formation, which can damage cells and cause cell death.

Note: DMSO is known to facilitate the entry of organic molecules into tissues. Handle reagents containing DMSO using equipment and practices appropriate for the hazards posed by such materials. Dispose of the reagents in compliance with local regulations.

Guidelines for Cryopreservation

Following the guidelines below is essential for cryopreserving your cell lines for future use. **As with other cell culture procedures, we recommend that you closely follow the instructions provided with your cell line for best results.**

- Freeze your cultured cells at a high concentration and at as low a passage number as possible. Make sure that the cells are at least 90% viable before freezing. Note that the optimal freezing conditions depend on the cell line in use.
- Freeze the cells slowly by reducing the temperature at approximately 1°C per minute using a controlled rate cryo-freezer or a cryo-freezing container such as "Mr. Frosty," available from NALGENE® labware (Nalge Nunc)
- Always use the recommended freezing medium. The freezing medium should contain a cryoprotective agent such as DMSO or glycerol (see **What is Subculture?**, page 26).
- Store the frozen cells below –70°C; frozen cells begin to deteriorate above –50°C.
- Always use sterile cryovials for storing frozen cells. Cryovials containing the frozen cells may be stored immersed in liquid nitrogen or in the gas phase above the liquid nitrogen (see Safety Note, page 37).
- Always wear personal protective equipment.
- All solutions and equipment that come in contact with the cells must be sterile. Always use proper sterile technique and work in a laminar flow hood.

Safety Note

Biohazardous materials **must** be stored in the gas phase above the liquid nitrogen. Storing the sealed cryovials in the gas phase eliminates the risk of explosion. If you are using liquid-phase storage, be aware of the explosion hazard with both glass and plastic cryovials and always wear a face shield or goggles.

Freezing Medium

Always use the recommended freezing medium for cryopreserving your cells. The freezing medium should contain a cryoprotective agent such as DMSO or glycerol. You may also use a specially formulated complete cryopreservation medium such as Recovery™ Cell Culture Freezing Medium or Synth-a-Freeze® Cryopreservation Medium.

- Recovery™ Cell Culture Freezing Medium is a ready-to-use complete cryopreservation medium for mammalian cell cultures, containing an optimized ratio of fetal bovine serum to bovine serum for improved cell viability and cell recovery after thawing.
- Synth-a-Freeze® Cryopreservation Medium is a chemically defined, protein-free, sterile cryopreservation medium containing 10% DMSO that is suitable for the cryopreservation of many stem and primary cell types with the exception of melanocytes.

Materials Needed

- Culture vessels containing cultured cells in log-phase of growth
- Complete growth medium
- Cryoprotective agent such as DMSO (use a bottle set aside for cell culture; open only in a laminar flow hood) or a freezing medium such as Synth-a-Freeze® Cryopreservation Medium or Recovery™ Cell Culture Freezing Medium
- Disposable, sterile 15-mL or 50-mL conical tubes
- Reagents and equipment to determine viable and total cell counts (e.g., Countess® Automated Cell Counter, or hemacytometer, cell counter and Trypan Blue)
- Sterile cryogenic storage vials (i.e., cryovials)
- Controlled rate freezing apparatus or isopropanol chamber
- Liquid nitrogen storage container

For freezing adherent cells, in addition to the above materials, you need:

- Balanced salt solution such as Dulbecco's Phosphate Buffered Saline (D-PBS), containing no calcium, magnesium, or phenol red
- Dissociation reagent such as trypsin or TrypLE™ Express, without phenol red

Protocol for Cryopreserving Cultured Cells

The following protocol describes a **general procedure** for cryopreserving cultured cells. **For detailed protocols, always refer to the cell-specific product insert.**

1. Prepare freezing medium and store at 2° to 8°C until use. Note that the appropriate freezing medium depends on the cell line.
2. For adherent cells, gently detach cells from the tissue culture vessel following the procedure used during the subculture. Resuspend the cells in complete medium required for that cell type.
3. Determine the total number of cells and percent viability using a hemacytometer, cell counter and Trypan Blue exclusion, or the Countess® Automated Cell Counter. According to the desired viable cell density, calculate the required volume of freezing medium.
4. Centrifuge the cell suspension at approximately 100–200 × *g* for 5 to 10 minutes Aseptically decant supernatant without disturbing the cell pellet.

   Note: Centrifugation speed and duration varies depending on the cell type.

5. Resuspend the cell pellet in cold freezing medium at the recommended viable cell density for the specific cell type.
6. Dispense aliquots of the cell suspension into cryogenic storage vials. As you aliquot them, frequently and gently mix the cells to maintain a homogeneous cell suspension.
7. Freeze the cells in a controlled rate freezing apparatus, decreasing the temperature approximately 1°C per minute. Alternatively, place the cryovials containing the cells in an isopropanol chamber and store them at –80°C overnight.
8. Transfer frozen cells to liquid nitrogen, and store them in the gas phase above the liquid nitrogen.

Thawing Frozen Cells

Guidelines for Thawing

The thawing procedure is stressful to frozen cells, and using good technique and working quickly ensures that a high proportion of the cells survive the procedure. As with other cell culture procedures, we recommend that you closely follow the instructions provided with your cells and other reagents for best results.

- Thaw frozen cells rapidly (< 1 minute) in a 37°C water bath.
- Dilute the thawed cells slowly, using pre-warmed growth medium.
- Plate thawed cells at high density to optimize recovery.
- Always use proper aseptic technique and work in a laminar flow hood.
- Always wear personal protective equipment, including a face mask or goggles. Cryovials stored in liquid-phase present a risk of explosion when thawed.
- Some freezing media contain DMSO, which is known to facilitate the entry of organic molecules into tissues. Handle reagents containing DMSO using equipment and practices appropriate for the hazards posed by such materials.

Materials Needed

- Cryovial containing frozen cells
- Complete growth medium, pre-warmed to 37°C
- Disposable, sterile centrifuge tubes
- Water bath at 37°C
- 70% ethanol
- Tissue-culture treated flasks, plates, or dishes

Protocol for Thawing Frozen Cells

The following protocol describes a **general procedure** for thawing cryopreserved cells. **For detailed protocols, always refer to the cell-specific product insert.**

1. Remove the cryovial containing the frozen cells from liquid nitrogen storage and **immediately** place it into a 37°C water bath.
2. Quickly thaw the cells (< 1 minute) by gently swirling the vial in the 37°C water bath until there is just a small bit of ice left in the vial.
3. Transfer the vial it into a laminar flow hood. Before opening, wipe the outside of the vial with 70% ethanol.
4. Transfer the thawed cells **dropwise** into the centrifuge tube containing the desired amount of pre-warmed complete growth medium appropriate for your cell line.
5. Centrifuge the cell suspension at approximately 200 × *g* for 5–10 minutes. The actual centrifugation speed and duration varies depending on the cell type.
6. After the centrifugation, check the clarity of supernatant and visibility of a complete pellet. Aseptically decant the supernatant without disturbing the cell pellet.
7. Gently resuspend the cells in complete growth medium, and transfer them into the appropriate culture vessel and into the recommended culture environment.

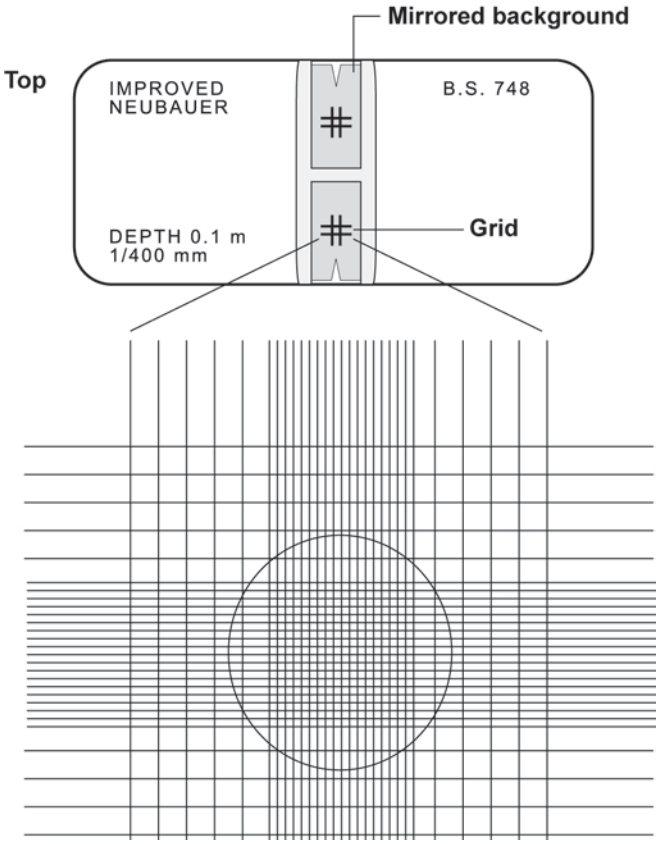
   Note: The appropriate flask size depends on the number of cells frozen in the cryovial, and the culture environment varies based on the cell and media type.

Support Protocols

Counting Cells in a Hemacytometer

Hemacytometers may be obtained from most major laboratory suppliers (e.g., Baxter Scientific). The procedure below provides some general directions on how to use the hemacytometer.

1. Clean the chamber and cover slip with alcohol. Dry and fix the coverslip in position.
2. Harvest the cells. Add 10 µL of the cells to the hemacytometer. Do not overfill.
3. Place the chamber in the inverted microscope under a 10X objective. Use phase contrast to distinguish the cells.
4. Count the cells in the large, central gridded square (1 mm^2). The gridded square is circled in the graphic below. Multiply by 10^4 to estimate the number of cells per mL. Prepare duplicate samples and average the count.



Trypan Blue Exclusion

The following procedure will enable you to accurately determine the cell viability. Cell viability is calculated as the number of viable cells divided by the total number of cells within the grids on the hemacytometer. If cells take up trypan blue, they are considered non-viable.

1. Determine the cell density of your cell line suspension using a hemacytometer.
2. Prepare a 0.4% solution of trypan blue in buffered isotonic salt solution, pH 7.2 to 7.3 (i.e., phosphate-buffered saline).
3. Add 0.1 mL of trypan blue stock solution to 1 mL of cells.
4. Load a hemacytometer and examine immediately under a microscope at low magnification.
5. Count the number of blue staining cells and the number of total cells. Cell viability should be at least 95% for healthy log-phase cultures.

   % viable cells = [1.00 – (Number of blue cells ÷ Number of total cells)] × 100

   To calculate the number of viable cells per mL of culture, use the formula below. Remember to correct for the dilution factor.

   Number of viable cells × 10^4 × 1.1 = cells/mL culture

Concentrating Cells

To concentrate cells from a suspension culture (or resuspended cells from monolayer culture):

1. Transfer the cell suspension to a sterile centrifuge tube of appropriate size and centrifuge for 10 minutes at 800 × g.

   Note: Certain cell lines are very sensitive to centrifugal force.

2. Carefully remove the supernatant without disturbing the cell pellet.
3. Add the desired volume of fresh medium gently to the side of the tube and slowly pipette up and down 2 to 3 times to resuspend the cell pellet.
4. Transfer the cells to the desired, sterile container.

Appendix

Troubleshooting

The table below lists some potential problems and possible solutions that may help you troubleshoot your cell culture experiments. Note that the list below includes only the most commonly encountered problems in cell culture, and provides guidelines to solutions only. To help evaluate your results more successfully, we recommend that you consult the manuals and product information sheets provided with the products you are using as well as the published literature and books on the subject.

Problem	Reason	Solution
No viable cells after thawing stock	Cells were stored incorrectly	Obtain new stock and store in liquid nitrogen. Keep the cells in liquid nitrogen until thawing.
	Home made freezer stock is not viable	Freeze cells at a density recommended by the supplier.
		Use low-passage cells to make your own freezer stocks.
		Follow procedures for freezing cells **exactly** as recommended by the supplier. Note that the freezing procedure recommended in this handbook is a general procedure provided as a guideline only.
		Obtain new stock.
	Cells were thawed incorrectly	Follow procedures for thawing cells **exactly** as recommended by the supplier. Note that the thawing procedure recommended in this handbook is a general procedure provided as a guideline only.
		Make sure that you thaw the frozen cells quickly, but dilute them slowly using pre-warmed growth medium before plating.
	Thawing medium is not correct	Use the medium recommended by the supplier. Make sure the medium is pre-warmed.
	Cells are too dilute	Plate thawed cells at high density as recommended by the supplier to optimize recovery.
	Cells not handled gently	Freezing and thawing procedures are stressful to most cells. Do **not** vortex, bang the flasks to dislodge the cells (except when culturing insect cells), or centrifuge the cells at high speeds.
	Glycerol used in the freezing medium was stored in light (if applicable)	If stored in light, glycerol is gets converted to acrolein, which toxic to cells. Obtain new stock.
Cells grow slowly	Growth medium is not correct	Use pre-warmed growth medium as recommended by the supplier.
	Serum in the growth medium is of poor quality	Use serum from a different lot.
	Cells have been passaged too many times	Use healthy, low passage-number cells.
	Cells were allowed to grow beyond confluency	Passage mammalian cells when they are in the log-phase before they reach confluence.
	Culture is contaminated with mycoplasma	Discard cells, media, and reagents. Obtain new stock of cells, and use them with fresh media and reagents.

Cell Culture Products

Life Technologies offers a variety of primary cultures and established cell lines, as well as reagents, media, sera, and growth factors for your cell culture experiments. The tables below contain lists of the more commonly used cell lines and other cell culture products available from Invitrogen, part of Life Technologies. For more information on Invitrogen and GIBCO® products, refer to www.invitrogen.com/cellculture.

Cell Lines

In addition to the mammalian and insect cell lines listed below, Invitrogen offers primary mammalian cells and complete cell culture systems, including keratinocyte, fibroblast, melanocyte, hepatocyte, corneal and mammary epithelial, large vessel and microvascular endothelial, smooth muscle, and neuronal cell culture systems. For a comprehensive list of cells, technical resources and related technologies, visit www.invitrogen.com/cellculture.

Product	Quantity	Cat. no.
Mammalian Cell Lines		
293-F Cells, SFM adapted (7.5×10^6 cells)	1.5 mL	11625-019
293-H Cells, SFM adapted (7.5×10^6 cells)	1.5 mL	11631-017
293FT Cell Line	3×10^6 cells	R700-07
293A Cell Line	3×10^6 cells	R705-07
GripTite™ 293 MSR Cell line	1 kit	R795-07
CHO DG44 Cells (cGMP banked) and Media Kit	1 kit	A11000-01
Insect Cell Lines		
Sf9 Cells, adapted in Sf-900™ II SFM (1.5×10^7 cells)	1.5 mL	11496-015
Sf21 Cells, adapted in Sf-900™ II SFM (1.5×10^7 cells)	1.5 mL	11497-013
Mimic™ Sf9 Insect Cells (1×10^7 cells)	1 mL	12552-014
Sf9 Cells adapted in Sf-900™ III SFM (1.5×10^7 cells)	1 vial	12659-017
Sf21 Cells adapted in Sf-900™ III SFM (1.5×10^7 cells)	1 vial	12682-019
Sf9 Frozen Cells (Grace's media) (1×10^7 cells)	1 mL	B825-01
Sf21 Frozen Cells (Grace's media) (1×10^7 cells)	1 mL	B821-01
High Five™ Cells, adapted to Express Five® SFM (3×10^6 cells)	1 mL	B855-02

Media for Mammalian Cell Culture

Life Technologies provides you with all of your cell culture needs through its GIBCO® Cell Culture Media, and offers products to support the growth of a range of mammalian cell lines. All cell culture media products available from Life Technologies are tested for contamination, and guaranteed for their quality, safety, consistency, and regulatory compliance. In addition to the media listed below, Life Technologies offers a large selection of serum-free and specialized media for culturing primary cells, established cell lines, and stem cells, as well as for virus production, protein expression, stem cell differentiation, and cytogenetics. For more information and a complete list of cell culture media, visit www.invitrogen.com/cellculture.

Product*	Quantity†	Cat. no.
D-MEM (1X), liquid (high glucose with no glutamine)	500 mL	31053-028
D-MEM (1X), liquid (low glucose with no glutamine)	500 mL	11880-028
D-MEM (1X), liquid (high glucose with GlutaMAX™-I)	10 × 500 mL	32430-100
D-MEM (1X), liquid (low glucose with GlutaMAX™-I)	10 × 500 mL	21885-108
Advanced D-MEM (1X), liquid (high glucose with no glutamine)	10 × 500 mL	12491-023
D-MEM/F-12, liquid, 1:1 (with GlutaMAX™-I)	10 × 500 mL	31331-093
Advanced D-MEM/F-12, liquid, 1:1 (with no glutamine)	10 × 500 mL	12634-028
Minimum Essential Medium (MEM) (1X), liquid (with no glutamine)	10 × 500 mL	21090-055
Minimum Essential Medium (MEM) (1X), liquid (with GlutaMAX™-I)	10 × 500 mL	41090-093
Advanced MEM (Minimum Essential Medium) (1X), liquid (with no glutamine)	10 × 500 mL	12492-021
RPMI Medium 1640 (1X), liquid (with no glutamine)	500 mL	31870-025
RPMI Medium 1640 (1X), liquid (with GlutaMAX™-I)	10 × 500 mL	61870-044
Advanced RPMI Medium 1640 (1X), liquid (with no glutamine)	10 × 500 mL	12633-020
293 SFM II, liquid	1,000 mL	11686-029
CD 293 Medium, liquid	1,000 mL	11913-019
GIBCO® Freestyle™ 293 Expression Medium	1,000 mL	12338-018
CD CHO Medium (1X), liquid	1,000 mL	10743-029
CHO-S-SFM II	1,000 mL	12052-098
GIBCO® Freestyle™ CHO Expression Medium	1,000 mL	12651-014
CD OptiCHO™ Medium (1X), liquid	1,000 mL	12681-011
Recovery™ Cell Culture Freezing Medium, liquid	50 mL	21530-027
Synth-a-Freeze® Cryopreservation Medium	50 mL	R-005-50

*Most of the media listed in this table are available with L-glutamine, GlutaMAX™-I, or no glutamine, with or without phenol red, as well as in powder and liquid formulations. †Also available in different quantities and packaging sizes.

Media for Insect Cell Culture

Insect cell culture is a common choice for heterologous protein expression. For large scale production or basic research, insect cells are able to express large quantities of protein with complex post-translational modifications. GIBCO® insect media from Life Technologies have been formulated for maximum growth and protein yields. For more information, visit www.invitrogen.com.

Product*	Quantity†	Cat. no.
Grace's Insect Cell Medium, Unsupplemented (1X), liquid	500 mL	11595-030
Grace's Insect Cell Medium, Supplemented (1X), liquid	500 mL	11605-045
Sf-900™ II SFM (1X), liquid	500 mL	10902-096
Sf-900™ III SFM (1X), liquid	500 mL	12658-019
Schneider's *Drosophila* Medium (1X), liquid	500 mL	21720-024
IPL-41 Insect Medium (1X), liquid	500 mL	11405-057
Express Five® SFM (1X), liquid	1,000 mL	10486-025

*Most of the media listed in this table are available in powder and liquid formulations. †Also available in different quantities and packaging sizes.

Serum Products for Cell Culture

Life Technologies supplies a wide range of GIBCO® animal sera, both bovine and non-bovine, for cell culture applications, the most widely used being fetal bovine serum (FBS). The table below lists a small selection of sera available from Life Technologies. For a complete list and more information on the use, sources, traceability, collection, and bottling of serum, refer to www.invitrogen.com.

Product	Source	Quantity	Cat. no.
Certified FBS	United States	100 mL 500 mL	16000-036 16000-044
Certified, heat-inactivated FBS	United States	100 mL 500 mL	10082-139 10082-147
Qualified FBS	United States	100 mL 500 mL	26140-087 26140-079
	Australia	100 mL 500 mL	10099-133 10099-141
	Countries that meet EU importation requirements (South America)	100 mL 500 mL	10270-098 10270-106
	Countries that meet EU importation requirements (excluding South America)	100 mL 500 mL	10106-151 10106-169
Qualified, heat-inactivated FBS	United States	100 mL 500 mL	16140-063 16140-071
	Australia	100 mL 500 mL	10100-139 10100-147
	Countries that meet EU importation requirements (South America)	100 mL 500 mL	10500-056 10500-064
	Countries that meet EU importation requirements (excluding South America)	100 mL 500 mL	10108-157 10108-165
Qualified, gamma-irradiated FBS	Countries that meet EU importation requirements (including South America)	100 mL 500 mL	10499-036 10499-044
	Countries that meet EU importation requirements (excluding South America)	100 mL 500 mL	10109-155 10109-165
Horse Serum	New Zealand	100 mL 500 mL	16050-130 16050-122
Horse Serum, heat-inactivated	New Zealand	100 mL 500 mL	26050-070 26050-088
Porcine Serum	New Zealand	500 mL	26250-084
Rabbit Serum	United States	100 mL 500 mL	16120-099 16120-107
Goat Serum	New Zealand	100 mL 500 mL	16210-064 16210-072
Chicken Serum	New Zealand	500 mL	16110-082
Lamb Serum	New Zealand	500 mL	16070-096
Bovine Serum	New Zealand	100 mL 500 mL	16170-086 16170-078

Laboratory Reagents for Cell Culture

The table below lists a small sampling of laboratory reagents for cell culture that are available from Life Technologies. For more information and a complete list, refer to www.invitrogen.com.

Product	Quantity*	Cat. no.
Balanced Salt Solutions: D-PBS†, EBSS, HBSS‡, PBS		
Dulbecco's Phosphate Buffered Saline (D-PBS) (1X), liquid	1,000 mL	14190-136
Earle's Balanced Salt Solution (EBSS) (1X), liquid	500 mL	14155-063
Hank's Balanced Salt Solution (HBSS) (1X), liquid	1,000 mL	14025-076
Phosphate-Buffered Saline (PBS) pH 7.4 (1X), liquid	500 mL	10010-023
Phosphate-Buffered Saline (PBS) pH 7.2 (1X), liquid	500 mL	70013-032
Buffers and Chemicals		
HEPES Buffer Solution (1M)	20 × 100 mL	15630-130
Sodium Bicarbonate Solution, 7.5% (w/v)	100 mL	25080-094
Cell Dissociation Reagents		
TrypLE™ Express Dissociation Reagent with Phenol Red	500 mL	12605-028
TrypLE™ Express Dissociation Reagent without Phenol Red	100 mL	12604-013
TrypLE™ Select Dissociation Reagent	500 mL	12563-029
Trypsin, 0.5% (10X), liquid, with EDTA 4Na, without Phenol Red	100 mL	15400-054
Trypsin, 0.25% (10X), liquid, without EDTA, with Phenol Red	500 mL	15050-057
Collagenase Type I	1 g	17100-017
Collagenase Type II	1 g	17101-015
Dispase	5 g	17105-041
Trypsin Inhibitor, soybean	1 g	17075-029
Supplements		
L-Glutamine†, 200 mM (100X), liquid	100 mL	25030-081
GlutaMAX™-I Supplement	100 mL	35050-061
D-Glucose (Dextrose)	1 kg	15023-021
Pluronic® F-68, 10% (100X)	100 mL	24040-032
MEM Amino Acids Solution (50X), liquid	100 mL	11130-051
MEM Non-Essential Amino Acids Solution 10 mM (100X), liquid	100 mL	11140-050
MEM Sodium Pyruvate Solution 10 mM (100X), liquid	100 mL	11360-070
MEM Vitamin Solution (100X), liquid	100 mL	11120-052
2-Mercaptoethanol (1,000X), liquid	50 mL	21985-023

*Products are also available in different quantities and packaging sizes. †Product is available in liquid or powder formats. ‡HBSS is available with or without magnesium and calcium, and with or without phenol red.

Antibiotics and Antimycotics

Antibiotics are used to protect the integrity of your cell culture as well for selection and establishment of cell lines; Life Technologies offers a wide selection of antibiotics, antimycotics and detection kits. For more information, refer to www.invitrogen.com.

Product	Quantity*	Cat. no.
Antibiotics and Antimycotics		
Antibiotic-Antimycotic (100X), liquid	100 mL	15240-062
Fungizone® Antimycotic, liquid	20 mL	15290-018
Gentamycin Reagent Solution (10 mg/mL), liquid	10 mL	15710-064
Gentamycin Reagent Solution (50 mg/mL), liquid	10 mL	15750-060
Gentamycin/Amphotericin Solution	10 × 1 mL	R-015-10
Neomycin Sulfate, powder	100 g	21810-031
Penicillin-Streptomycin, liquid	100 mL	15140-122
Penicillin-Streptomycin-Neomycin (PSN) Antibiotic Mixture	100 mL	15640-055
Selection Antibiotics		
Geneticin® Selective Antibiotic, liquid	20 mL	10131-027
Geneticin® Selective Antibiotic, powder	1 g	11811-023
Hygromycin B	20 mL	10687-010
Puromycin Dihydrochloride, Selection Antibiotic, liquid	10 × 1 mL	A11138-03
Blasticidin S HCl, Selection Antibiotic, liquid	20 mL	A11139-02
Blasticidin S HCl, powder	50 mg	R210-01
Zeocin™ Selection Reagent, powder	1 g	R250-01
Contamination Detection Kits		
Cell Culture Contamination Detection Kit - 200 assays	1 kit	C7028
MycoFluor™ Mycoplasma Detection Kit	1 kit	M7006
*Products are also available in different quantities and packaging sizes.		

Accessory Products for Cell Culture

The table below lists a small sampling of accessory products for cell culture that are available from Life Technologies. For more information and a complete list, refer to www.invitrogen.com.

Product	Quantity*	Cat. no.
Attachment Factors and Matrices		
Geltrex™ Reduced Growth Factor Basement Membrane Matrix	5 mL	12760-021
Geltrex™ LDEV-free Reduced Growth Factor Basement Membrane Matrix Extract	5 mL	A11343-01
Geltrex™ hESC-qualified Reduced Growth Factor Basement Membrane Matrix	1 mL 5 mL	A10480-01 A10480-02
AlgiMatrix™ 3D culture System 6-well plates	1 plate	A10982-01
AlgiMatrix™ 3D culture System 24-well plates	1 plate	12684-023
AlgiMatrix™ 3D culture System 96-well plates	1 plate	12684-015
Human Plasma Fibronectin	5 mg	33016-015
Natural Mouse Laminin	1 mg	23017-015
Collagen I, Rat Tail	20 mL	A1048301
Collagen I, Bovine	10 mL	A10644-01
Collagen I, Coated Plate 6-well	5 plates	A11428-01
Collagen I, Coated Plate 24-well	5 plates	A11428-02
Collagen I, Coated Plate 96-well	5 plates	A11428-03
Instruments		
Countess® Automated Cell Counter (includes 50 Countess® cell counting chamber slides and 2 mL of Trypan Blue Stain)	1 unit	C10227
Mallassez Hemocytometer	1 unit	99503
Neon™ Transfection System	1 unit	MPK5000
Qubit® Fluorometer	1 unit	Q32857
*Some of the products listed in the table are also available in different quantities and packaging sizes.		

Growth Factors and Purified Proteins

Life Technologies offers an array of highly-potent and highly-pure growth factors, chemokines, cytokines, and other proteins and protein inhibitors validated for use in cell culture. These products have been validated in live cell bioassays using GIBCO® media. For more information and a complete list, refer to www.invitrogen.com/cellculture.

Transfection and Selection

Researchers have a wide variety of gene delivery techniques to introduce plasmid DNA, siRNA or duplex RNAi, oligonucleotides, and RNA into eukaryotic cells for a variety of research and drug discovery applications. For more information on the most appropriate transfection method for your cell line and application, refer to our **Transfection Selection Guide**.

Plasmid Transfection

Life Technologies offers the most complete collection of cationic lipid-based transfection reagents with exceptional performance that can be used for plasmid delivery into a broad range of cells. Lipofectamine™ LTX with PLUS™ Reagent (Cat. no. 15338-100) is a plasmid DNA-specific transfection reagent that provides maximum expression with minimum cytotoxicity.

RNAi Transfection

RNA interference (RNAi) technology is revolutionizing the biological discovery process as well as target discovery and validation. Using RNAi, you can turn gene expression "off", or knock it down, to better understand its function and role in disease. High-efficiency transfection is an essential first step for achieving effective gene knockdown. Life Technologies offers the most complete collection of cationic lipid-based transfection reagents with exceptional performance that can be used for delivery of assorted RNAi reagents, including shRNA and miR RNAi vectors and synthetic molecules such as siRNA, Stealth RNAi™ siRNA, and Dicer-generated siRNAi pools. Further, cell specific RNAi transfection protocols have been developed using these transfection reagents for many popular cell lines. For more information and a complete list, refer to www.invitrogen.com/transfection.

Transient Protein Production and High Throughput Screening

For the highest protein yields in mammalian suspension cells, FreeStyle™ MAX Transfection Reagent (Cat. no. 16447-100) is a versatile and scalable transient expression tool for CHO and 293 suspension cell cultures. FreeStyle™ MAX Reagent is a proprietary, animal origin-free formulation for the transfection of plasmid DNA into eukaryotic cells that can easily be scaled up to produce large amounts of recombinant proteins. FreeStyle™ MAX Reagent allows the highest expression levels and transfection rates with lowest cytotoxicity in bio-production applications.

Viral Delivery Systems

Having evolved to proficiently deliver nucleic acids to cells, viruses offer a means to reach hard-to-transfect cell types for protein overexpression or knockdown. Adenoviral, oncoretroviral, and lentiviral vectors have been used extensively for delivery in cell culture and *in vivo*. Life Technologies offers a variety of products used in viral-mediated transduction, including BLOCK-iT™ Adenoviral and Lentiviral Expression Systems. For more information, refer to www.invitrogen.com.

Neon™ Transfection System

The Neon™ Transfection System (Cat. no. MPK5000) is a novel, benchtop electroporation device that employs an electroporation technology by using the pipette tip as an electroporation chamber to efficiently transfect mammalian cells including primary and immortalized hematopoietic cells, stem cells, and primary cells. The Neon™ Transfection System efficiently delivers nucleic acids, proteins, and siRNA into all mammalian cell types including primary and stem cells with a high cell survival rate.

Transfection Reagents

For most applications, including plasmid DNA and siRNA transfections, Lipofectamine™ 2000 Transfection Reagent provides the best efficiency and gene expression with maximum viability across a broad range of adherent and suspension cell lines. In addition to Lipofectamine™ 2000, Life Technologies has additional reagents which may better suit your experiments. Consult the information below for your particular application.

Transfection Reagent	Cat. no.	Key Features and Applications of Reagent
Lipofectamine™ LTX	15338-500	• Provides high transfection efficiency and significantly lower toxicity levels for a wide range of cell lines • Provides significantly improved transfection performance in a number of primary and hard-to-transfect cell lines • Optimized protocols for over 30 different cell lines are available so much less time is needed for evaluation and optimization
Lipofectamine™ LTX and PLUS Reagent	15338-100	
FreeStyle™ MAX	16447-100 16447-500 16447-750	• Optimized for transient transfection in CHO suspension cells and also works for HEK-293 cells • Used for a large-scale transient protein production with milligrams of protein yield
Lipofectamine™ RNAiMAX	13778-075 13778-150	• Superior transfection efficiency requiring lower RNAi concentrations leading to more effective gene knockdown with minimal non-specific effects • Easy optimization due to minimal cytotoxicity across a 10-fold concentration range of transfection reagent • Compatibility with a broad range of cell types providing the most versatile approach to all of your gene silencing experiments
Lipofectamine™ 2000	11668-019 11668-027 11668-500	• Achieves highest efficiency and expression results for plasmid or RNAi transfections • Works effectively with many cell types (adherent or suspension) • Easy and fast protocol – no need for wash steps before or after transfection • Complexes can be added to cells growing in serum-containing media • Ideal if transfecting at confluencies of 90% or greater (minimizes cytotoxicity following transfection)
Lipofectamine™ 2000 CD	12566-014	Same performance as Lipofectamine™ 2000, certified animal-origin free ("CD" = chemically defined)
Oligofectamine™	12252-011	Transfection of antisense oligonucleotides
293fectin™	12347-019 12347-500 12347-750	• Used for transient protein production in combination with the FreeStyle™ 293 Expression System • Optimized for suspension FreeStyle™ 293-F cells
Cellfectin® II	10362-100	Optimal transfection of insect cells, including S2, Sf9, Sf21 and High Five™ cells
DMRIE-C	10459-014	Transfection of suspension cells, including CHO, lymphoid and Jurkat cell lines
Optifect™	12579-017	• Broad use reagent designed for low confluency applications (<70% confluent at the time of transfection) • Useful for cell lines that are sensitive to transfection reagents

Additional Resources

Mammalian and Insect Cell Cultures

For more information on mammalian and insect cell culture, cell type specific protocols, and additional cell culture products, refer to **Mammalian Cell Culture** and **Insect Cell Culture** portals on our website.

Cell and Tissue Analysis

Understanding the structural and functional relationships of cells and tissues is critical to advancements in key research disciplines, including molecular biology, genetics, reproductive function, immunology, cancer and neurobiology. Key components of cell and tissue analysis are cell viability and proliferation, cell signaling pathways, cell cycle analysis, and cell structure. Life Technologies has a broad portfolio of reagents and kits for cell and tissue analysis, including Molecular Probes® Fluorescence Products and technologies as well as Dynal® Bead-based Solutions for cell isolation and expansion. From antibodies and stem cell research products to benchtop instruments like the Countess™ Cell Counter and Qubit™ Fluorometer, Life Technologies has the tools essential for cellular analysis research. For more information, refer to the **Cell & Tissue Analysis** portal on our website.

Transfection Selection Tool

Transfection of DNA, siRNA, oligonucleotides, and RNA into mammalian cells is a critical step in many research efforts of the biomedical scientific community including gene silencing using RNAi, transient gene expression studies, protein/antibody production, and generation of stable cell lines. To simplify the transfection optimization process, Invitrogen, part of Life Technologies, has created a transfection selection tool that provides information on recommended transfection reagents with optimized protocols for over 120 cell lines. Invitrogen offers a wide range of transfection reagents for your specific needs. For more information and cell type specific protocols, refer to **Transfection Selection Tool** on our website.

Safety Data Sheets

Safety Data Sheets (SDS) are available at www.invitrogen.com/sds.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.invitrogen.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

Technical Support

World Wide Web

Visit the Invitrogen website at www.invitrogen.com for:

- Technical resources, including manuals, vector maps and sequences, application notes, SDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

Contact Us

For more information or technical assistance, call, write, fax, or e-mail. Additional international offices are listed on our website (www.invitrogen.com).

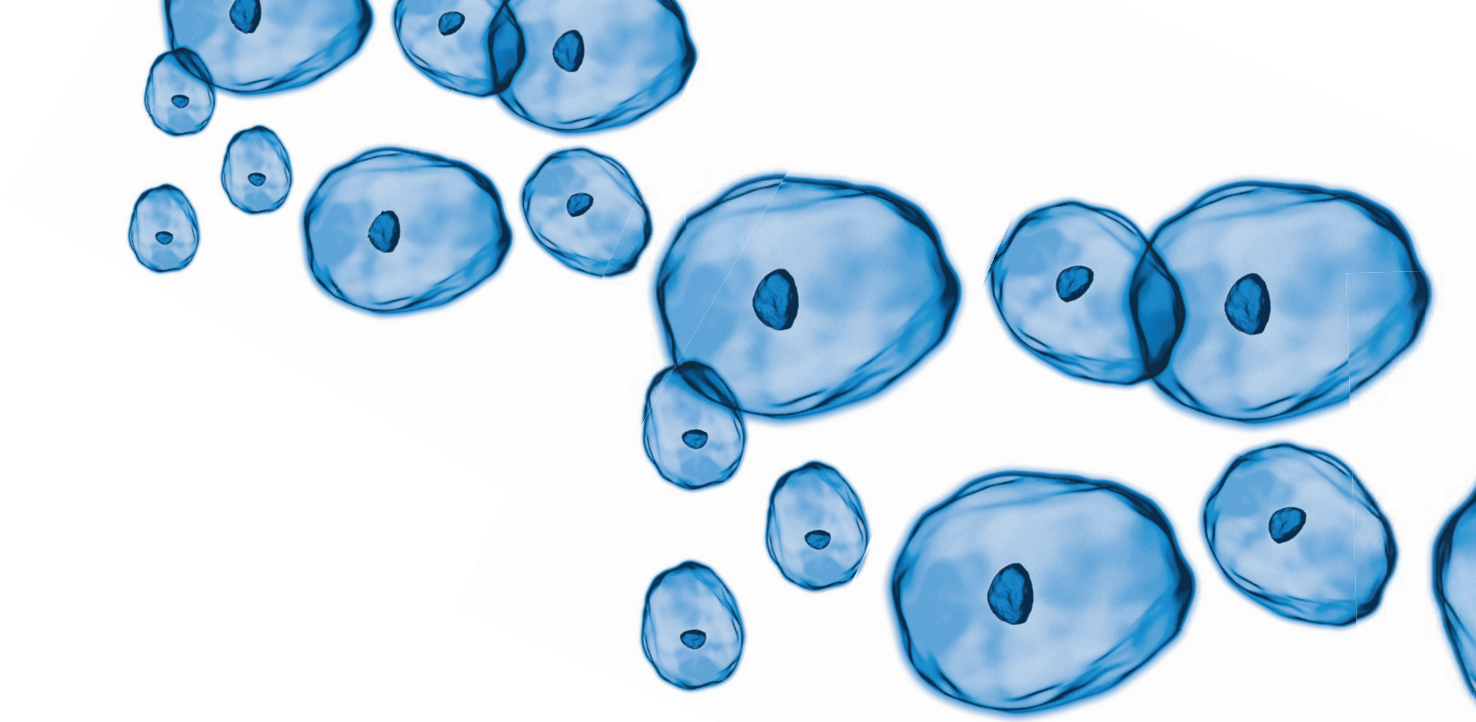
Corporate Headquarters	Japanese Headquarters	European Headquarters
5791 Van Allen Way Carlsbad, CA 92008 USA Tel: 1 760 603 7200 Tel (Toll Free): 1 800 955 6288 Fax: 1 760 602 6500 E-mail: tech_support@invitrogen.com	LOOP-X Bldg. 6F 3-9-15, Kaigan Minato-ku, Tokyo 108-0022 Tel: 81 3 5730 6509 Fax: 81 3 5730 6519 E-mail: jpinfo@invitrogen.com	Inchinnan Business Park 3 Fountain Drive Paisley PA4 9RF, UK Tel: +44 (0) 141 814 6100 Tech Fax: +44 (0) 141 814 6117 E-mail: eurotech@invitrogen.com

Limited Warranty

Invitrogen (a part of Life Technologies Corporation) is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, contact our Technical Support Representatives. All Invitrogen products are warranted to perform according to specifications stated on the certificate of analysis. The Company will replace, free of charge, any product that does not meet those specifications. **This warranty limits the Company's liability to only the price of the product.** No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. The Company reserves the right to select the method(s) used to analyze a product unless the Company agrees to a specified method in writing prior to acceptance of the order. Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore the Company makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Support Representatives.

Life Technologies Corporation shall have no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.

Notes



invitrogen™ | GIBCO®

www.invitrogen.com/cellculturebasics

B-087243 0110