

# Getting Started with Cell Culture

Tools & Techniques for Success at the Bench

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# About Us



ATCC is a global leader in providing authenticated, high-quality biological resources and standards for industry, academia, and government.

- Founded in 1925, ATCC is a private, nonprofit, global biological resource center and standards organization that provides scientists with the biomaterials and resources they need to conduct critical life science research.
- World's trusted, premier biological materials resource and standards development organization:
  - 4,000+ cell lines
  - 80,000+ microorganisms
  - Genomic and synthetic nucleic acids
  - Media, sera, and reagents
  - Advanced cell models
  - Standards



# Agenda



Initiation



Expansion



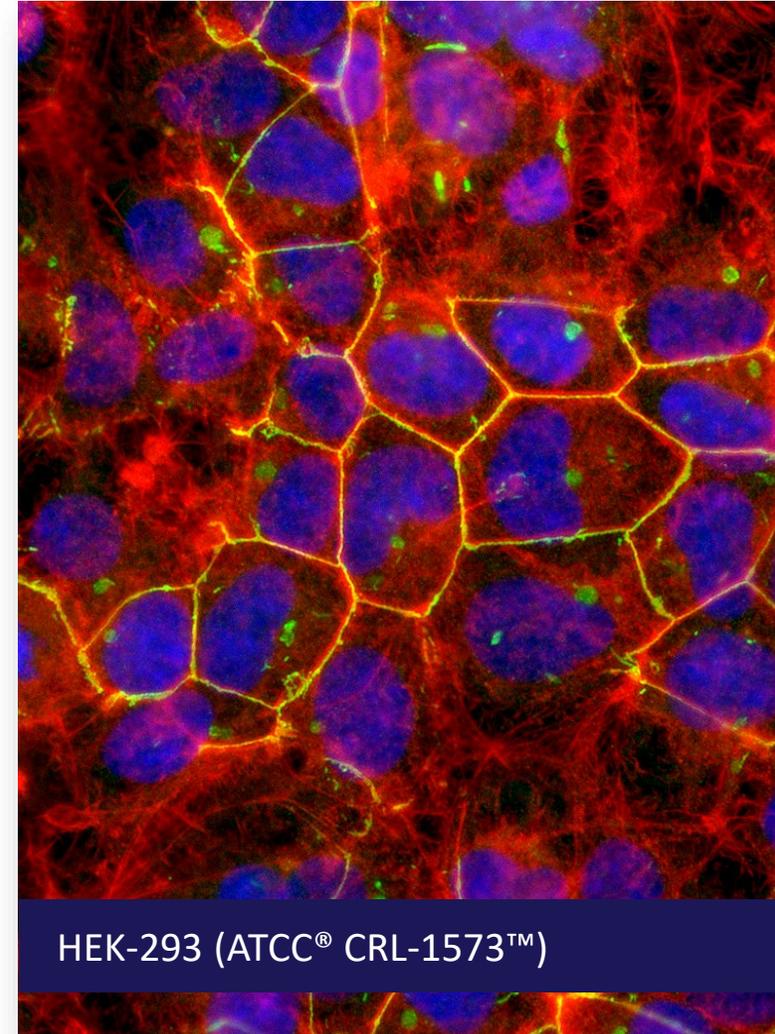
Cryopreservation



Authentication



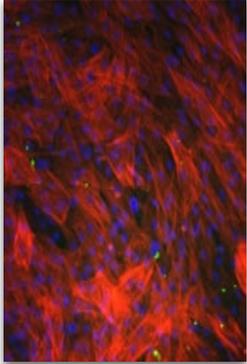
Troubleshooting



# Initiation

Understanding how the biological origin and type of culture dictate the seeding, expansion, and recovery process

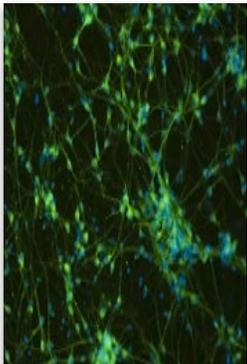
# Selecting Your Model



**Primary Cells:** Cells harvested directly from tissue.

**Considerations:**

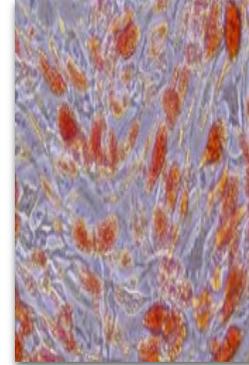
- More physiologically relevant.
- Finite number passages.
- Very sensitive to growth conditions.



**Induced Pluripotent Stem Cells:** Human somatic cells that have been genetically reprogrammed to become embryonic stem cell-like.

**Considerations:**

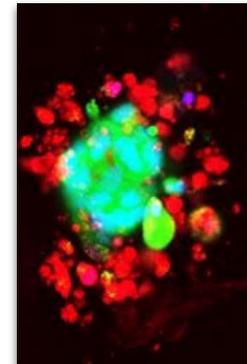
- Ability to differentiate into specific lineages.
- Require feeder-dependent or independent cell culture systems.



**Continuous Cells:** Cells that generally do not senesce and instead proliferate for a long time in culture.

**Considerations:**

- Generally resilient and can proliferate in non-specialize conditions.
- Prone to genetic drift over as passage number increases.

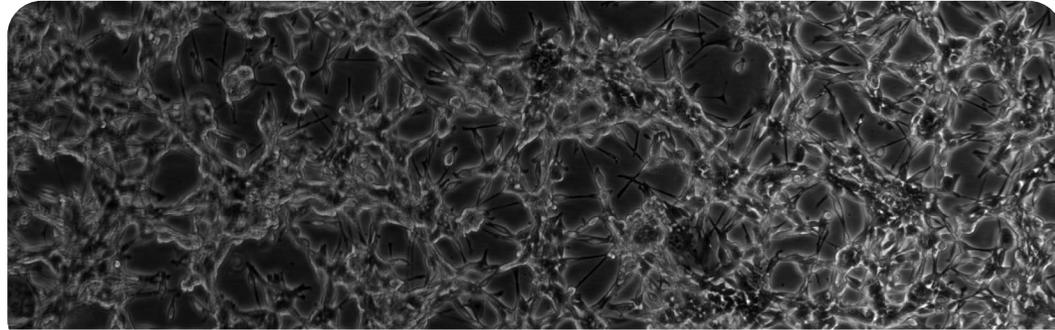


**Organoids:** Micro 3-D tissues contained within an extracellular matrix

**Considerations**

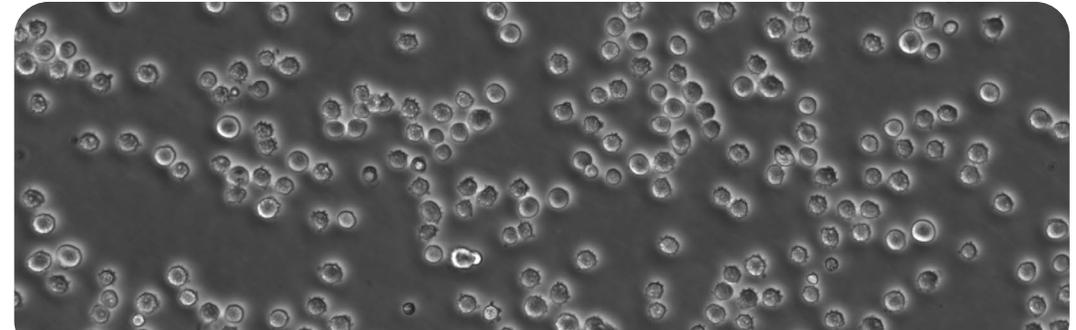
- Most physiologically relevant.
- Require lineage-specific growth factors.
- Most complex to culture.

# Adherent versus Suspension



## Adherent Cell Cultures

- Cells requiring attachment to a solid substrate for growth.
- Considerations:
  - Growth limited by surface area
  - Passaging requires detachment from surface
  - Lag in growth is impacted by attachment rate
  - Do I need to pre-coating with collagen, fibronectin, or laminin?



## Suspension Cell Cultures

- Cells that grow in liquid media without attachment.
- Considerations:
  - Growth limited by nutrient availability, oxygen transfer, and cell density
  - Require agitation for oxygen/nutrient distribution

# Classical Basal Media



- Commonly used for human and animal cells lines
- Contain amino acids, vitamins, salts, and other components
- Certain cells are adapted to the media they require:
  - ATCC immune cells often use RPMI-1640
  - Cells requiring ambient CO<sub>2</sub> use L-15

**ATCC manufactures formulations of these media to support the growth and maintenance of its cell lines**



ATCC® number	Product name
30-2001™	RPMI-1640 Medium
30-2002™	Dulbecco's Modified Eagle's Medium (DMEM)
30-2003™	Eagles Minimum Essential Medium (EMEM)
30-2004™	Kaighn's Modification of Ham's F-12 Medium (F-12K)
30-2005™	Iscove's Modified Dulbecco's Medium (IMDM)
30-2006™	DMEM: F-12K
30-2007™	McCoy's 5a Medium
30-2008™	Leibovitz's L-15 Medium

# Animal Serum



- Used as an addition to cell culture media (2 – 10%) to make complete growth media
- Add needed growth hormones and supplements needed for growth
- Fetal Bovine and Calf Serum are most common and popular serum used in human and animal culture.
- ATCC FBS is from USDA approved origins

**ATCC manufactures cell lines with ATCC serum**



ATCC® number	Product Name
30-2020™	Fetal Bovine Serum (FBS) – 500 mL
30-2021™	Fetal Bovine Serum (FBS) – 100 mL
SCRR-30-2020™	Fetal Bovine Serum (FBS) - ES Cell Qualified
30-2030™	Calf Bovine Serum (CBS) – 500 mL
30-2031™	Calf Bovine Serum (CBS) – 100 mL

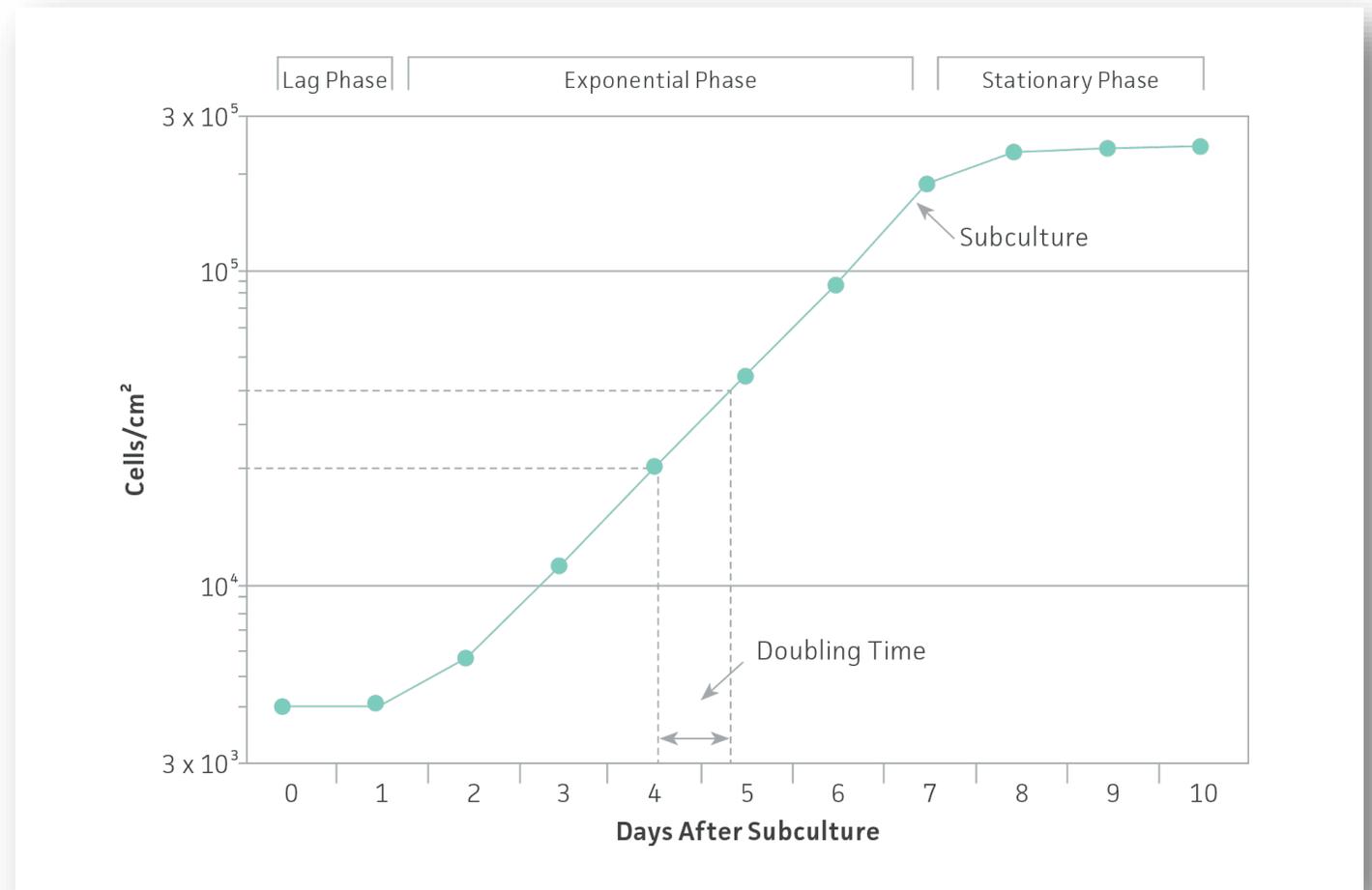
# Expansion

Scaling up cultures through  
subculturing and maintenance

# Monitoring Growth

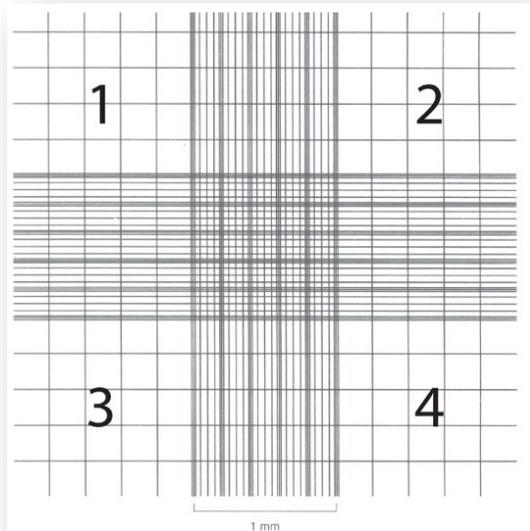


- Cultures should be checked routinely for **confluence**, **morphology**, and **pH** changes (color shifts in phenol red), and **viability**.
- Cell growth falls within 4 major stages
  - **Lag Phase**
  - **Exponential Phase**
  - **Stationary Phase**
  - **Decline Phase**



# Cell Count & Characterization

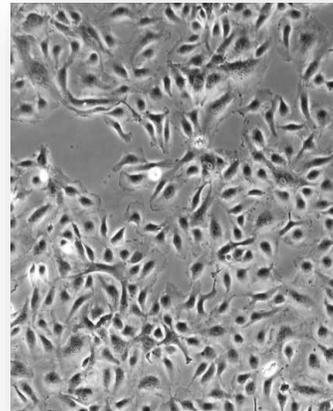
## Hemocytometer



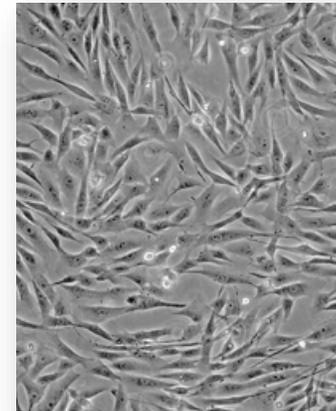
## Calculating % viability

1. Transfer small amount of cell suspension
2. Place under inverted microscope at 100X magnification
3. Count cells in each quadrant (1-4)
4. Multiply by the dilution factor

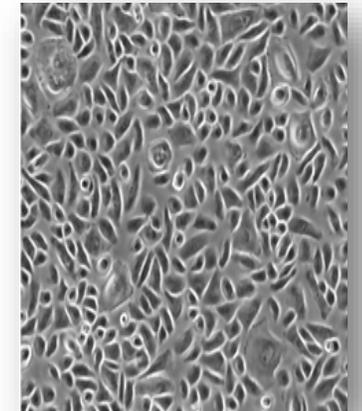
## Cell Morphology



Endothelial Cells  
Elongated



Fibroblasts  
Elongated and spindle shaped

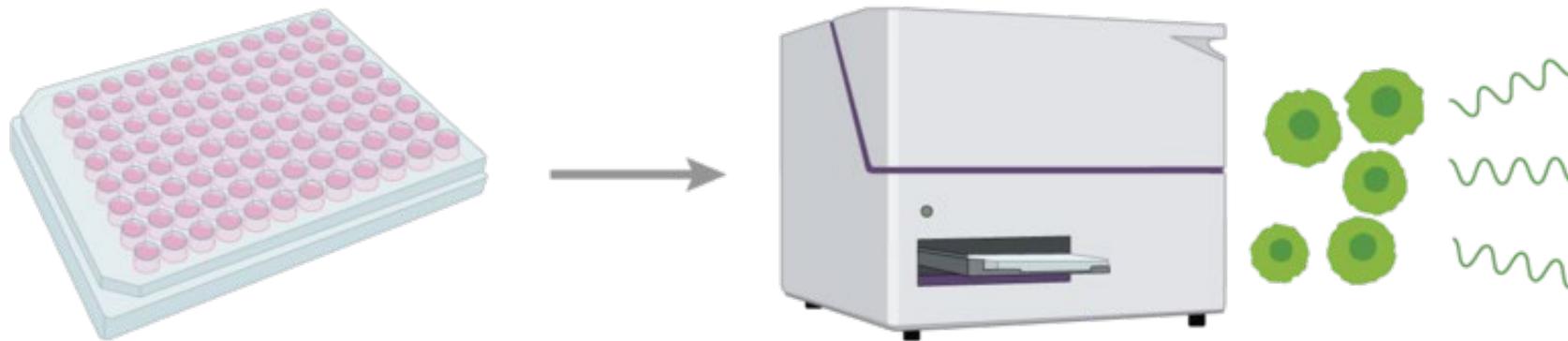


Epithelial Cells  
Cobblestone

# Viability Assays

Quantitative evaluation of cell proliferation rate and response to external factors that affect cell viability

- Commonly used for cytotoxicity, high-throughput screening (e.g., drug development)
- Uses tetrazolium salts in a colorimetric method for evaluating cell populations

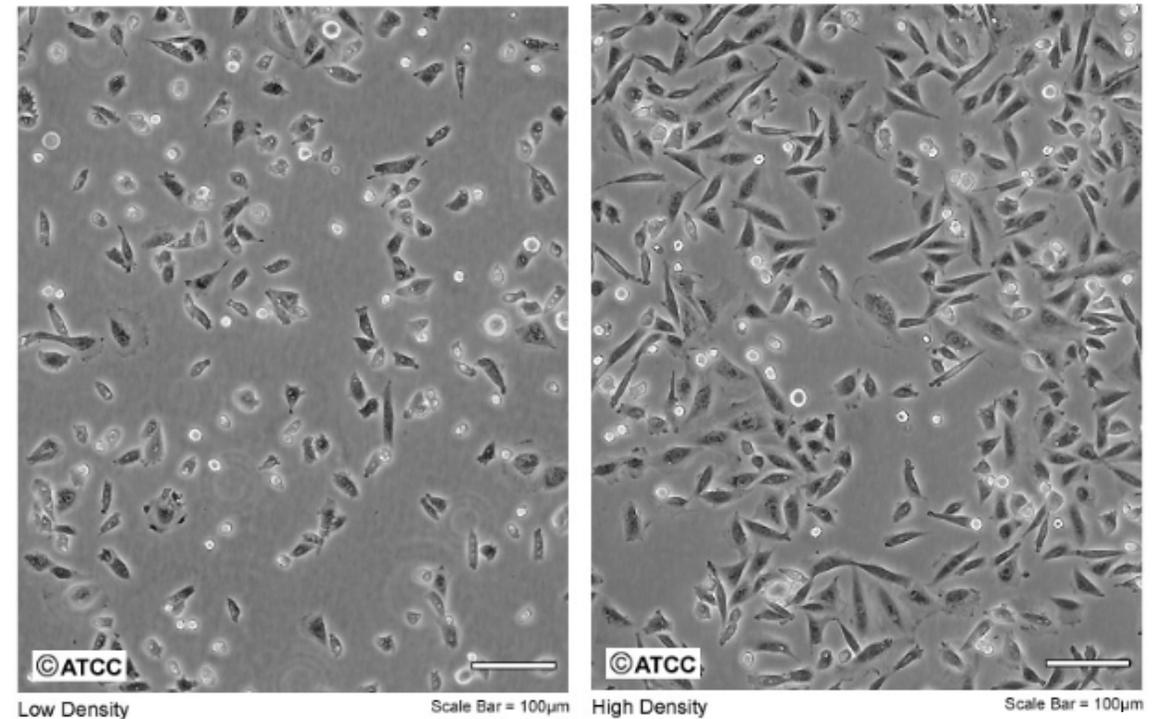


ATCC® number	Product name
30-1010K™	MTT Cell Proliferation Assay

# When to Subculture

- As cell density (i.e., confluency for adherent cultures) increases, cells will reach a point where they will need to be subcultured
- Subculturing is the process of taking a portion within a container moving them to a separate container
- This serves several purposes:
  - Preventing overcrowding
  - Replenishes nutrients

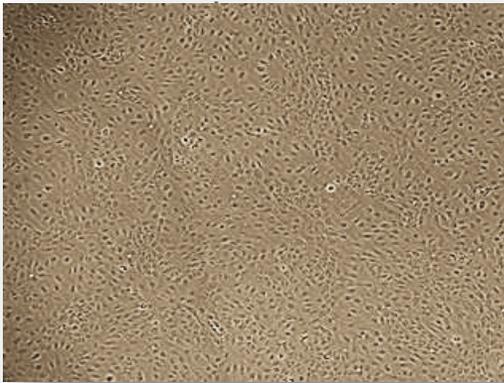
## CHO-K1 (ATCC® CCL-61™)



ATCC® number	Product name
30-2200™	Dulbecco's Phosphate Buffered Saline (D-PBS)
30-2213™	Hank's Balanced Salt Solution (HBSS)

# Trypsinization

Cells are passaged using Trypsin-EDTA (or non-enzymatic)



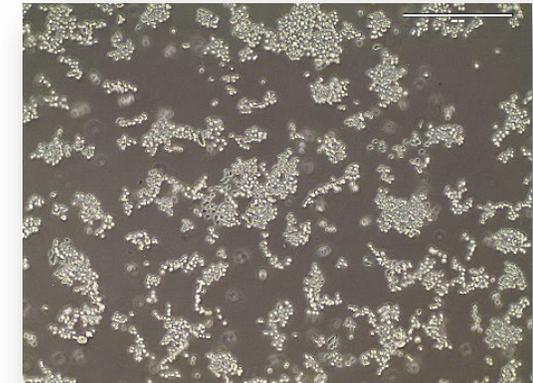
**Passage 1**  
Monolayer

Using warm Trypsin-EDTA for 3-5 minutes will detach cells with gentle agitation



Once trypsinized, the cell passage number increases

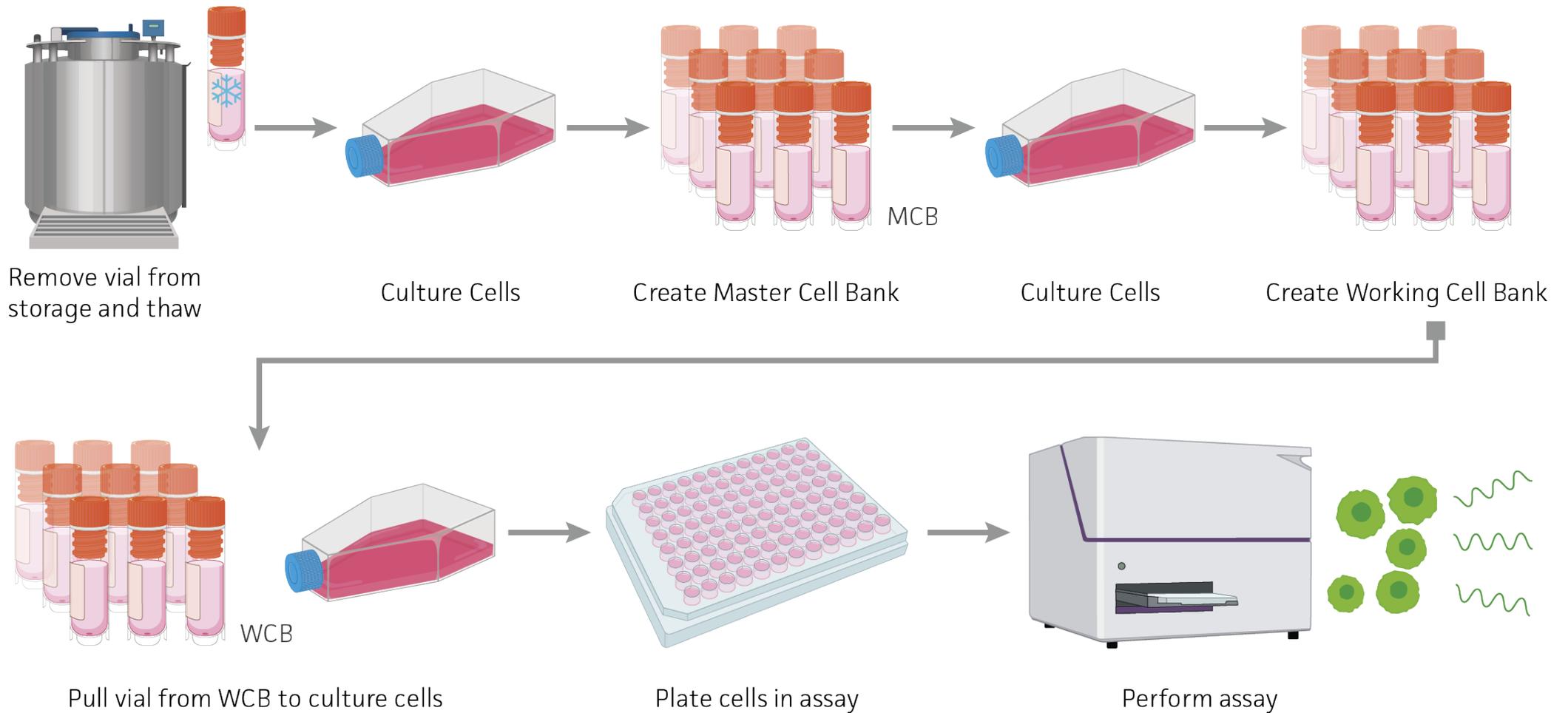
A neutralizing solution is needed to prevent cell damage and allow reattachment



**Passage 2**  
Fully trypsinized

ATCC® number	Product name
30-2101™	Trypsin-EDTA - .25% Trypsin / .53 mM EDTA
PCS-999-003™	Trypsin-EDTA for Primary Cells - .05% Trypsin / .02% EDTA
30-2103™	Non-Enzymatic Cell Dissociation Solution
20-2104™	Trypsin Soybean Neutralizing Solution

# Master and Working Cell Banks



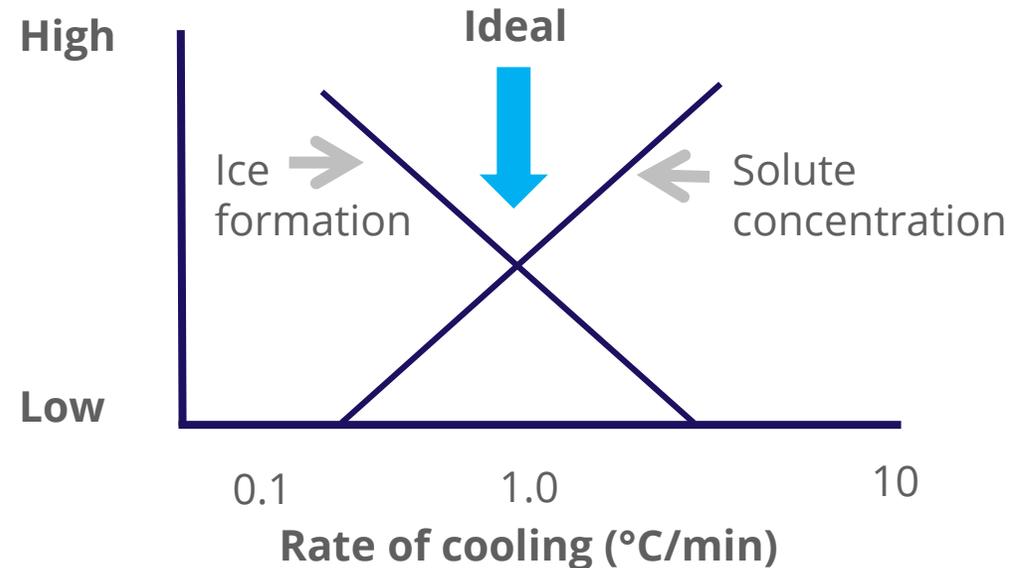
# Cryopreservation

Preserving the genotypic and phenotypic integrity of stocks for long-term use

# Cryoprotectants & Controlled Cooling



- Most cell cultures can be stored for many years, if not indefinitely, using cryopreservation. Most cryopreservation techniques store cells at temperatures below  $-140^{\circ}\text{C}$ .
  - At these temperatures, all metabolic activity has been arrested.
  - The very low temperatures structurally preserve living cells and tissues that could be damaged when using regular freezing methods.
- High levels of ice formation and increased solute concentration have a negative impact on cell viability.
- Optimal cooling rate for cell viability is 1 to  $3^{\circ}\text{C}/\text{min}$ .



ATCC® number	Product name
4-X™	Dimethylsulfoxide (DMSO)
ACS-3020™	Serum Free Freezing Media
ACS-6000™	CoolCell LX Alcohol-Free Cryopreservation Container

# Long-Term Storage

-70°C

Controlled-rate freeze chamber

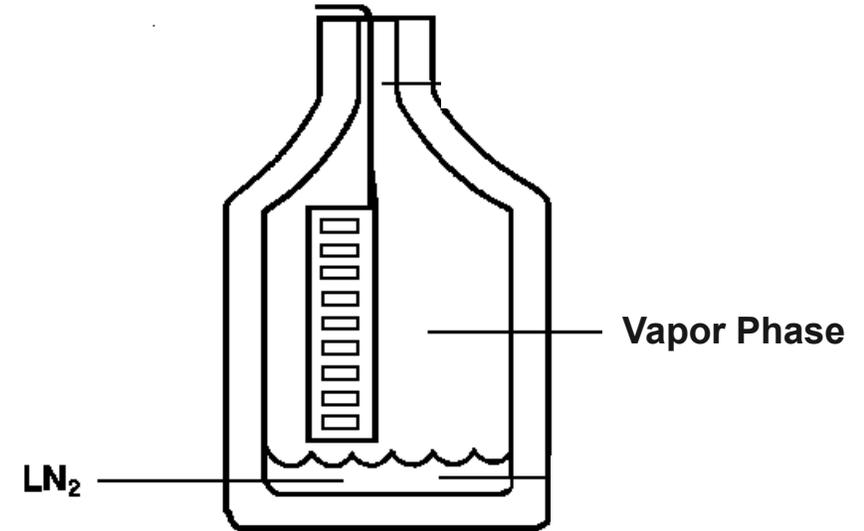
-1°C/min cooling rate

A few hours to 24 hours



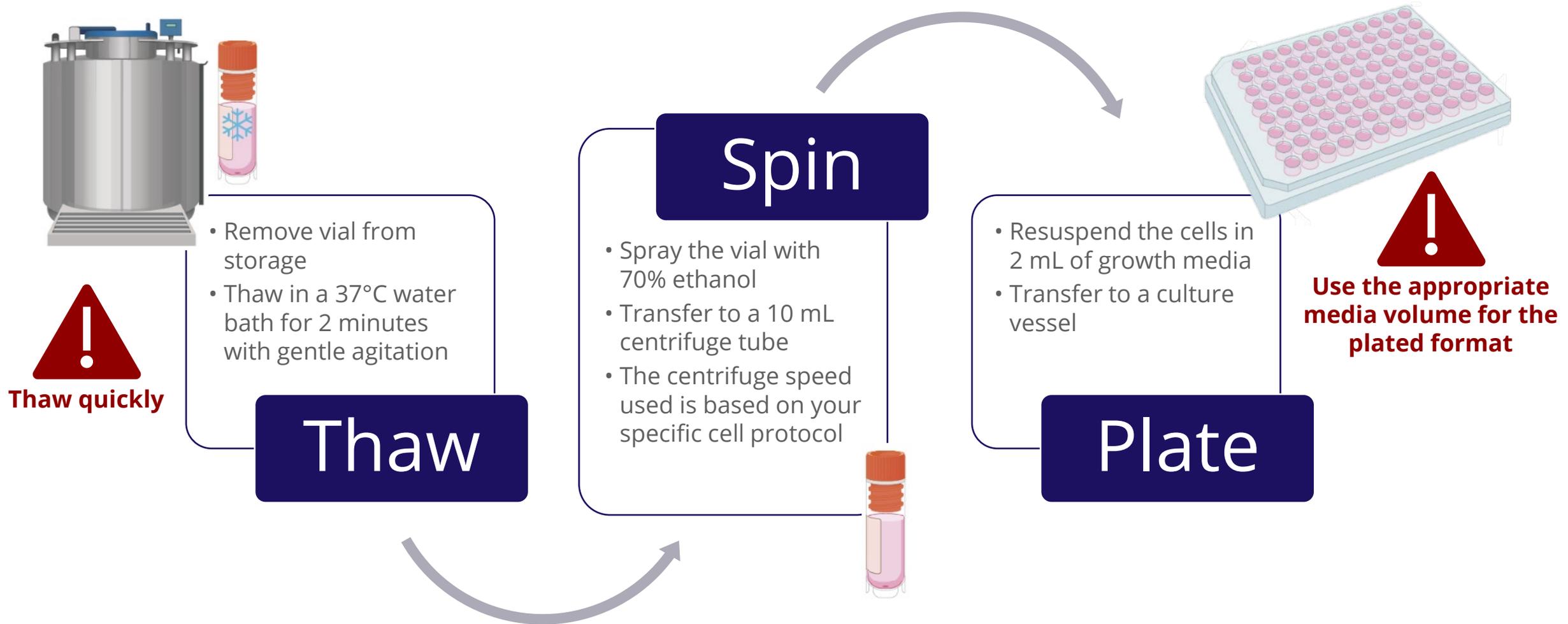
-140°C

Liquid nitrogen tank



- Long-term storage should be below -140°C
- Vials should be stored in a liquid nitrogen unit **above** the volume of liquid at the bottom of the tank – **Vapor Phase**
- This temperature should be between **-140°C** and **-180 °C**

# Thawing Cells



# Authentication

Confirming the identity of your material and preventing misidentification

# Cell Authentication

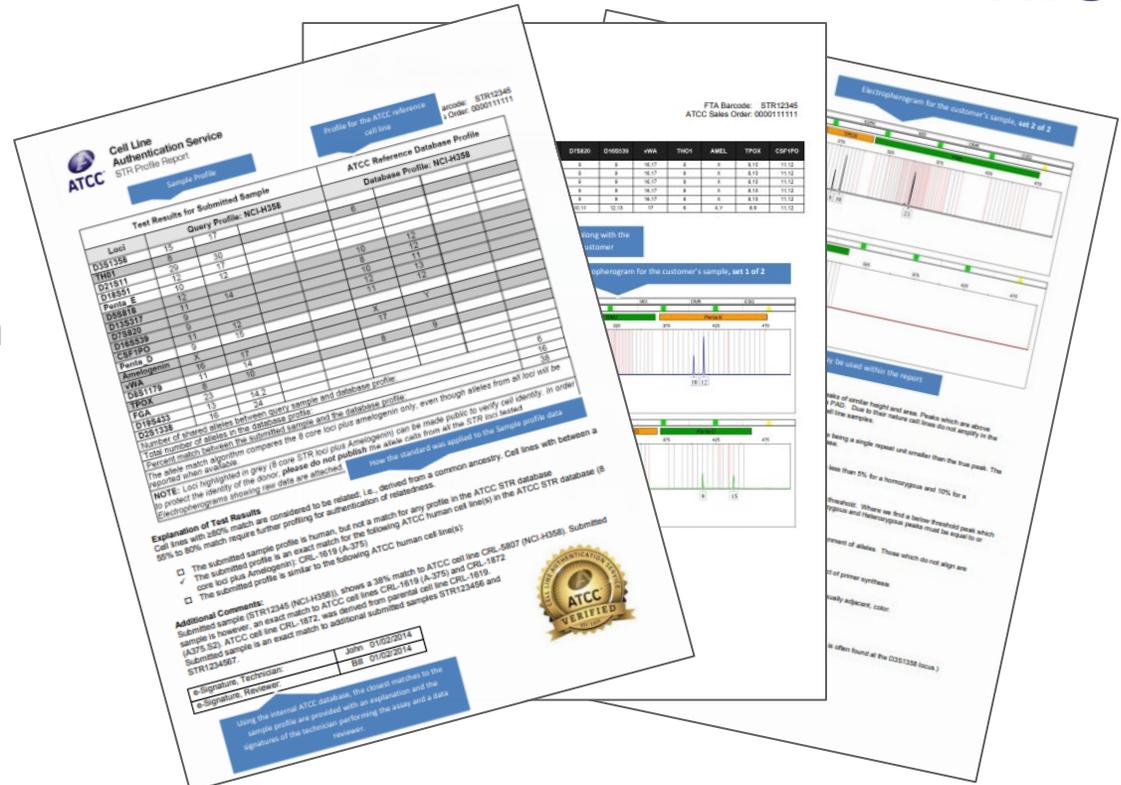


## Cell authentication service

- STR profiling for human and mouse cell lines
- Simple to use – spot on sample card and mail in
- Quick – receive results within 5 days

## Report includes:

- Submitted and matched allele calls
- Contamination check (to ensure no cross contamination from other cell lines)
- Comparative output for database comparison
- PDF of sample profile

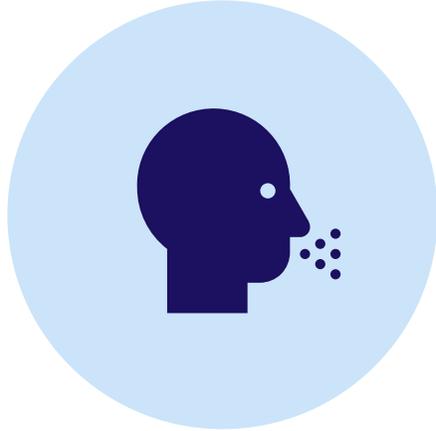


ATCC® number	Product name
135-XV™ 135-XV -10™ 135-XV-20™	Sample Collection Kit(s) for Human Cell Authentication Service (single, 10, or 20 kits)
137-XV™	Sample Collection Kit for Mouse Cell Authentication Service

# Troubleshooting

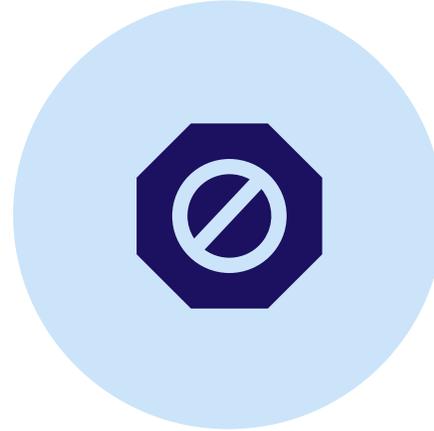
Identifying, managing, and preventing  
biological and chemical contaminants

# Contamination in Cultures



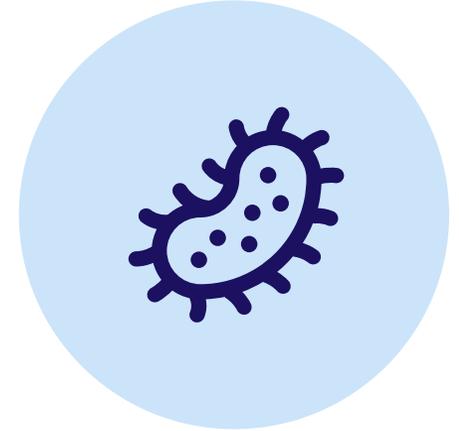
## Sources

- Contaminated cell lines
- Improper aseptic technique
- Dust and aerosols
- Faulty laminar flow
- Contaminated media/reagents



## Signs

- Turbid media
- Decline in pH
- Morphological changes
- Filamentous structures
- Changes in physiological responses



## Types

- Bacteria
- Mycoplasma
- Fungi
- Viruses
- Cross-contamination

# Bacterial and Fungal Contamination



## Personnel and equipment

- Poor culturing practices
- Dust and aerosol



## Contamination

- Aerosol dispersion of contaminated cell cultures
- Faulty laminar flow



## Culture reagents

- Sera
- Media
- Reagents



# Mycoplasma Contamination

## Mycoplasma contamination is not easy to detect

- No media turbidity
- No pH alternation
- No detection by light microscopy

## Deleterious effects

- Chromosomal aberrations
- Inhibition of cell proliferation and metabolism
- Decreased transfection rates
- Affects virus production
- Cell death



# Mycoplasma Testing



## Universal Mycoplasma Detection Kit

- Detects over 120 species of Mycoplasma
- Quick, sensitive PCR-based test
- All components required for the PCR reaction are provided and optimized for amplification



## PCR-based Mycoplasma Detection Service

- Detects over 120 species of Mycoplasma
- Easy to use – spot sample on collection card
- Fast – 3-5 business days for results

ATCC® number	Product name
136-XV™	Sample Collection Kit for PCR-based Mycoplasma Detection Service
30-1012K™	Mycoplasma Detection Kit

# Contamination Prevention & Aseptic Technique



## Make it difficult for microorganisms to invade culture vessels

- Sealed cultured vessels
- Vented cap flasks
- Use disposable aspirators

## Maintain your biological cabinet

- Cell culture hoods with good laminar flow
- Avoid clutter – do not use as a storage area

## Clean your area and equipment

- Clean incubator and water bad regularly
- Spray hood with alcohol after each use
- Spray media bottles/reagents with alcohol when entering the hood



# Contamination Prevention & Aseptic Technique



## Avoid contaminating bulk material

- Use small volumes of reagents at a time
- Create master stocks of cells

## Use personnel protective gear

- Gloves cleaned with alcohol
- Clean lab coats

## Do not rely on antibiotics!

- Can prolong mycoplasma contamination
- Lead to false sense of security



# Summary

# Initiation Summary



## Initiation

Cell culturing is not a one-size-fits-all process. Researching the model is the understand the key metrics for health and the conditions in which a model can be grown.

ATCC® number	Product name	Description
30-2001™	RPMI-1640 Medium	ATCC®-formulated classical media are sterile, ready-to-use cell culture medium optimized for the growth of a wide variety of mammalian cell lines.  Cell culture medium is dependent on cell line and application.
30-2002™	Dulbecco's Modified Eagle's Medium (DMEM)	
30-2003™	Eagles Minimum Essential Medium (EMEM)	
30-2004™	Kaighn's Modification of Ham's F-12 Medium (F-12K)	
30-2005™	Iscove's Modified Dulbecco's Medium (IMDM)	
30-2006™	DMEM: F-12K	
30-2007™	McCoy's 5a Medium	
30-2008™	Leibovitz's L-15 Medium	

# Initiation Summary (Continued)



## Initiation

The cell model will determine the reagents needed for growth. ATCC provides guides for every model offered by ATCC.

ATCC® number	Product name	Description
30-2020™	Fetal Bovine Serum (FBS) – 500 mL	Serum supplement providing growth factors, hormones, and proteins necessary for robust cell proliferation.
30-2021™	Fetal Bovine Serum (FBS) – 100 mL	
SCRR-30-2020™	Fetal Bovine Serum (FBS) - ES Cell Qualified	Serum supplement tested for its ability to support the growth and maintenance of embryonic stem cells in an undifferentiated state.
30-2030	Calf Bovine Serum (CBS) – 500 mL™	Serum supplement suitable for less fastidious cell lines, providing baseline proteins and nutrients.
30-2031™	Calf Bovine Serum (CBS – 100 mL	
30-1010K™	MTT Cell Proliferation Assay	Colorimetric assay that measures cell metabolic activity as an indicator of viability and proliferation.
30-2020™	Fetal Bovine Serum (FBS) – 500 mL	Serum supplement providing growth factors, hormones, and proteins necessary for robust cell proliferation.
30-2021™	Fetal Bovine Serum (FBS) – 100 mL	

# Expansion Summary



## Expansion

Monitor key growth characteristics to determine when your cultures need to be passaged and when nutrients need to be replenished.

ATCC® number	Product name	Description
30-2200™	Dulbecco's Phosphate Buffered Saline (D-PBS)	Buffered salt solution used for washing cells, preparing suspensions, and maintaining isotonic conditions during manipulation.
30-2213™	Hank's Balanced Salt Solution (HBSS)	A balanced salt buffer ideal for washing and handling cells
30-2101™	Trypsin-EDTA - .25% Trypsin / .53 mM EDTA	Enzymatic dissociation reagent for detaching adherent mammalian cells during routine passaging.
PCS-999-003™	Trypsin-EDTA for Primary Cells - .05% Trypsin / .02% EDTA	Gentler trypsin formulation designed to minimize damage when passaging sensitive primary cells.
30-2103™	Non-Enzymatic Cell Dissociation Solution	Chelator-based dissociation reagent used to lift fragile or enzyme-sensitive cell types without proteolytic stress.
20-2104™	Trypsin Soybean Neutralizing Solution	Neutralizing solution that stops trypsin activity following cell detachment, protecting cells from over-digestion.

# Cryopreservation Summary



## Cryopreservation

Remember the "Golden Rule": Cool at  $-1^{\circ}\text{C}$  per minute and to use cryoprotectants to minimize viability loss during freezing.

ATCC® number	Product name	Description
4-X™	Dimethylsulfoxide (DMSO)	ATCC's DMSO is a cell culture grade cryoprotectant for the cryopreservation of cell lines.
ACS-3020™	Stem Cell Freezing Media	Cryopreservation media specifically for the freezing of various types of human and non-human animal stem cells.
ACS-6000™	CoolCell® LX Alcohol-Free Cryopreservation Container	Alcohol-free Cryopreservation container can be used in cryopreservation leading to optimal cell viability.

# Authentication Summary



## Authentication

Remember to authenticate upon receipt, before banking, and before publication. Up to 15-30% of cell lines in literature may be misidentified.

ATCC® number	Product name	Description
135-XV™ 135-XV -10™ 135-XV-20™	Sample Collection Kit(s) for Human Cell Authentication Service (single, 10, or 20 kits)	Kit to collect sample human cell lines that will be sent to ATCC to confirm cell authentication utilizing Short Tandem Repeat (STR) profiling.
137-XV™	Sample Collection Kit for Mouse Cell Authentication Service	Kit to collect sample mouse cell lines that will be sent to ATCC to confirm cell authentication utilizing Short Tandem Repeat (STR) profiling.
136-XV™	Sample Collection Kit for PCR-based Mycoplasma Detection Service	Kit to collect culture sample that will be sent to ATCC to test for mycoplasma via polymerase chain reaction (PCR) testing.

# Troubleshooting Summary



## Troubleshooting

Aseptic techniques are key in minimizing contamination; but even with proper technique, be cognizant of contamination such as bacteria, fungi, viruses, and mycoplasma.

ATCC® 30-1012K™ : Mycoplasma Detection Kit

PCR-based test to detect mycoplasma contaminants in cell culture.

# Additional Supporting Resources

