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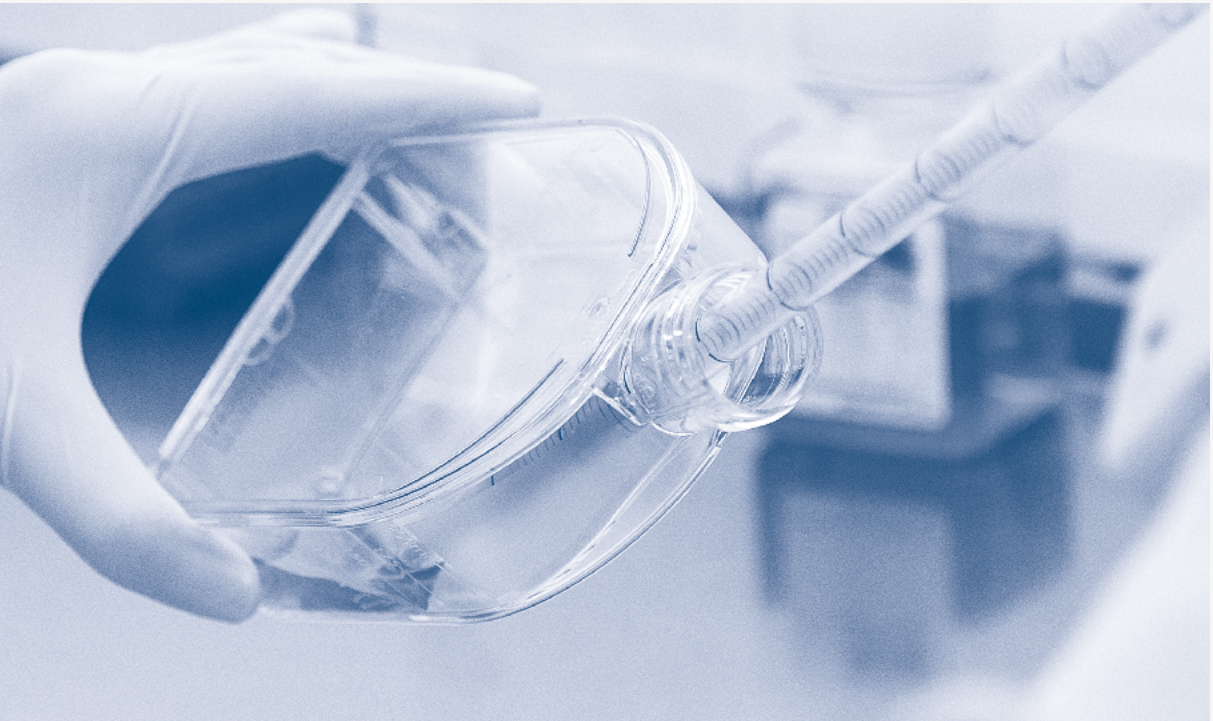
# CELL CULTURE BASICS

## Overview and Technical Tips



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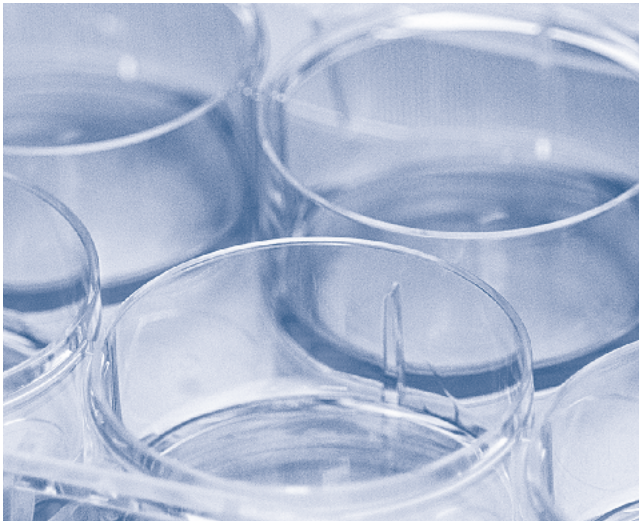
# CONTENTS





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# AT A GLANCE

## Definition

- **Removal of cells from animals/plants.**
- **Followed by cell growth in an artifical environment.**
- **Primary culture:**
  Refers to the initial stage of the culture after direct cell isolation.
- **Subcultures:**
  Refers to the stage of cells after passaging to a new vessel.

| Factors influencing cell culture conditions |
|---|
| Cell type |
| Cell vessel |
| Cell medium and additives |
| Growth factors/hormones |
| Gas regulation ($O_2$, $CO_2$) |
| Temperature |

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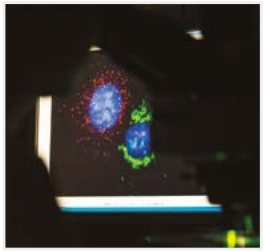
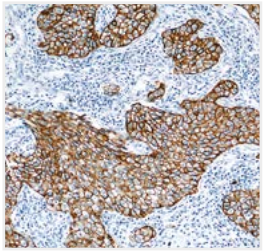
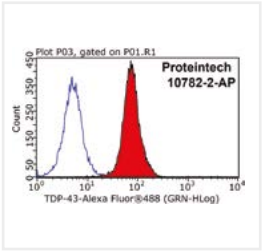
# APPLICATIONS

- Major tool in molecular and cellular biology.
- Helps to study normal cell homeostasis, cell biochemistry, metabolism, mutagenesis, diseases, compound effects.
- Model system for diseases and drug screening.
- Consistent and reproducible tool.

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# CELL CULTURE SAFETY LEVELS

**Work in a cell culture laboratory is associated with different risk factors and hazards:**

- Toxins
- Mutagenetic reagents
- Manipulating human/animal material

## Biosafety Levels

**Guidelines and recommendations for biosafety in cell culture laboratories describe the appropriate handling and practice, needed safety equipment, and facility infrastructure in order to work at a certain biosafety level.**

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# CELL CULTURE SAFETY LEVELS

## General Safety Laboratory Practices

- Wash hands before leaving the laboratory.
- Wear safety clothes (gloves, closed shoes, lab coat).
- No eating, drinking, smoking.
- No or low aerosol creation.
- Decontamination of all surfaces before and after the experiment.
- Work in accordance with the facility guidelines.
- Reporting all incidences to the safety officer.

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# CELL CULTURE EQUIPMENT

## Basic Equipment

- Cell culture hood
- Cell incubator
- Centrifuge
- Water bath
- Cell counter
- Fridge/freezer
- Autoclave
- Aspiratory pump
- Pipettes
- Cell culture vessels
- Media, sera, cell media additives
- Waste container
- Cells

**Please Note:**

- **The specific cell culture equipment depends on the cell type and aim of study.**

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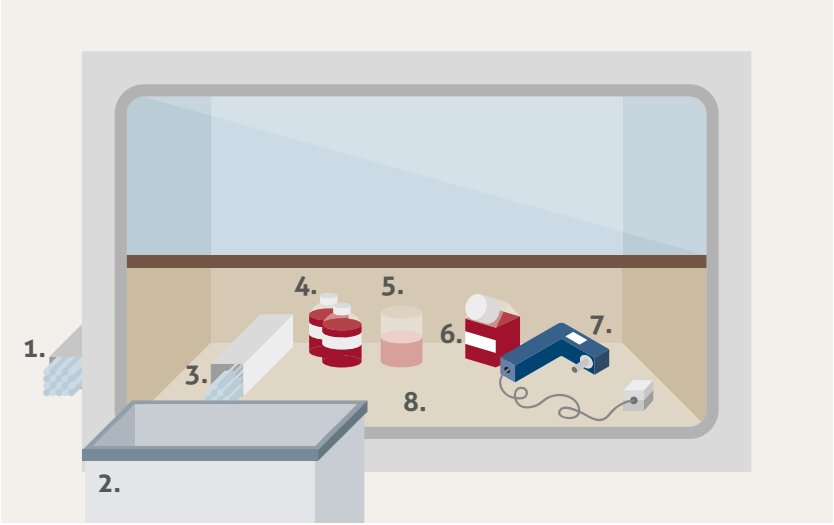
# CELL CULTURE EQUIPMENT




1. Wrapped disposable pipettes
2. Waste container
3. Glass pipettes (if using)
4. Cell culture flasks
5. Waste liquid
6. Reagents and media
7. Pipettor
8. Work surface

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# ASEPTIC TECHNIQUES

**To be successful in cell culture, it is essential to remain a contamination free environment (bacteria, fungi etc). Aseptic techniques ensure that no microorganisms enter the cell culture.**

**Cell culture sterility is ensured by a set of procedures:**

| Handling | Reagents/Media | Workplace |
|---|---|---|
| Slow/careful handling | Pre-sterilization of all reagents/equipment. | Cell culture hood works properly. |
| Sterilization of all items before starting. | No contamination in reagents (expiration date, appearance normal). | Frequent de-contamination (hood, fridge etc). |
| Sterile pipettes. | | Work area: sterile and tidy. |
| No touching of sterile items to non-sterilized surfaces. | | |

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# BIOLOGICAL CONTAMINATIONS

**Biological contaminations occur in laboratories if aseptic techniques are not carried out.**

## Major Biological Contaminations

- **Bacteria:**
  Large unicellular microorganisms, variety of shapes.
- **Yeast:**
  Unicellular eukaryotic microorganisms, spherical particles.
- **Mycoplasma:**
  Very small bacteria lacking a cell wall, difficult to detect.

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# CELL CULTURE ENVIRONMENT

**Cell culture is an amazing tool that allows for easy controlling and manipulation of all physiochemical and physiological cell factors, such as, temperature, osmotic pressure, pH, gas, hormones, and nutrients.**

| Media | pH | Temperature | $CO_2$ |
|---|---|---|---|
| • Contains nutrients, growth factors, and hormones.<br>• Sera: source of growth, lipids, hormones. | • Average pH for mammalian cells is pH 7.4. | • Depends on body temperature of host.<br>• Mammalian cell lines 36–37 °C.<br>• Insect cell lines 27–30°C. | • Controlled by media.<br>• Organic or $CO_2$ bicarbonate buffer systems are popular.<br>• Can impact pH.<br>• 4–10% $CO_2$ is most common. |

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# FREEZING & THAWING CELLS

## Freezing Cells

- **As soon as enough cells of the starting culture are available, stock aliquots should be prepared when passaging.**
- **Freezing cells at an early passage is essential as cells in culture always prone to contamination, senescence, and genetic shifts.**
- **Storage:**
  Liquid nitrogen, freezing media, DMSO.

## Thawing Cells

- **Thawing is very stressful for the cells.**
- **Working fast is essential.**
- **After thawing, cells should be passaged at least one time to ensure normal cell behaviour.**

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