

Why Are My Cells Not Growing?

Common Cell Culture Problems and Solutions

Introduction

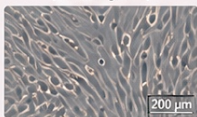
Cell culture is an essential technique in biomedical research, drug discovery, molecular biology, and biotechnology. However, maintaining healthy and actively growing cells can be challenging.

Researchers frequently encounter slow cell growth, reduced viability, contamination, unexpected changes in cell morphology, and inconsistent experimental results.

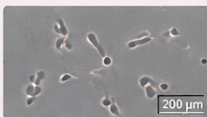
Understanding these problems and their underlying causes is essential for maintaining reliable cell culture conditions.

1 Slow growth or no growth

Healthy, proliferating cells (confluent growth)



Slow growth / poor proliferation

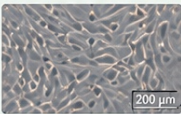


Common causes

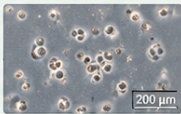
- Unsuitable medium or supplements
- Low serum quality
- Incorrect incubation conditions (temperature, CO₂, pH)
- Incorrect seeding density
- Cell senescence or stress
- Mycoplasma contamination

2 High cell death or low viability

Healthy, viable cells



High cell death (rounded cells)

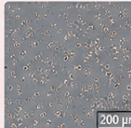


Common causes

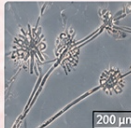
- Excessive trypsinization or harsh handling
- Inappropriate centrifugation
- Temperature or osmotic stress
- Poor thawing or freezing procedures
- Toxic compounds or reagents
- Unsuitable culture conditions

3 Contamination

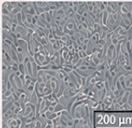
Bacterial contamination



Fungal contamination



Mycoplasma contamination (no visible turbidity)

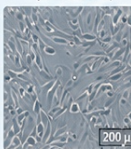


Common causes

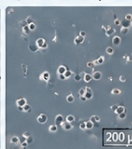
- Poor aseptic technique
- Contaminated media, serum or reagents
- Bacterial, fungal or mycoplasma contamination
- Cross-contamination between cell lines

4 Cells not attaching

Good attachment (adherent cells)



Poor attachment (most cells in suspension)

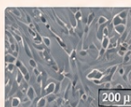


Common causes

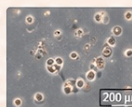
- Unsuitable culture surface
- Lack of extracellular matrix coating
- Poor cell viability
- Excessive trypsinization
- Inappropriate medium composition

5 Unexpected changes in cell morphology

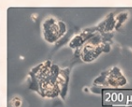
Normal morphology



Rounded and stressed cells



Enlarged / granular cells



Common causes

- Cellular stress
- Nutrient depletion
- Over-confluence
- Contamination
- Differentiation or phenotypic changes
- Genetic drift or prolonged passaging

6 Medium-related issues

pH change (color shift)



Normal pH (red/pink)



High pH (purple)

Precipitation in medium



Turbidity



Common causes

- Expired or improperly stored medium
- Contaminated serum or supplements
- Incorrect storage conditions
- pH changes due to over-incubation
- Precipitation from supplements
- Microbial contamination

Environmental conditions



37 °C
(temperature)



5%
(CO₂ concentration)



Humidity
(prevent evaporation)

1. Slow Cell Growth or No Cell Proliferation

The Problem

Cells remain viable but divide slowly, or their population fails to increase as expected.

Possible Causes

- Unsuitable culture medium or nutrient composition.
- Insufficient or inappropriate serum supplementation.
- Incorrect incubation temperature, CO₂ concentration, or medium pH.
- Excessive cell density or insufficient initial seeding density.
- Cells reaching replicative senescence or undergoing stress.
- Mycoplasma contamination.

Practical Solutions

Verify that the culture medium is suitable for the cell type. Check serum quality, incubation conditions, and the recommended seeding density.

Examine the cells under a microscope for changes in morphology and test for mycoplasma if growth remains unexpectedly slow.

Practical tip: Always compare growth performance against the established characteristics of the specific cell line. Different cell types have very different proliferation rates.

2. High Cell Death or Low Viability

The Problem

A large proportion of cells die after seeding, passaging, thawing, or treatment.

Possible Causes

- Excessive exposure to dissociation reagents such as trypsin.
- Inappropriate centrifugation conditions.
- Rapid temperature changes or osmotic stress.
- Poor freezing or thawing practices.
- Toxic concentrations of experimental compounds.
- Unsuitable culture conditions.

Practical Solutions

Use validated dissociation and handling procedures, minimize unnecessary manipulation, and assess cell viability after thawing or passaging.

For drug-treatment experiments, include untreated and vehicle controls to distinguish treatment-related toxicity from handling-related cell death.

3. Cell Culture Contamination

The Problem

Culture medium becomes cloudy, changes color unexpectedly, or contains visible particles or microorganisms.

Possible Causes

- Bacterial or fungal contamination.
- Mycoplasma contamination.
- Poor aseptic technique.
- Contaminated media, serum, or reagents.
- Cross-contamination between cell lines.

Practical Solutions

Inspect cultures regularly and follow established aseptic procedures. Use appropriate contamination-testing methods, including routine mycoplasma testing.

Confirmed contaminated cultures should be managed according to the laboratory's validated contamination-control procedures.

Important: Mycoplasma contamination may not produce visible turbidity. A culture can appear normal while its experimental behavior is significantly altered.

4. Cells Are Not Attaching to the Culture Surface

The Problem

Adherent cells remain suspended or attach poorly after seeding.

Possible Causes

- Unsuitable culture vessel surface.
- Insufficient extracellular matrix coating.
- Poor cell viability.
- Excessive enzymatic dissociation.
- Inappropriate medium composition.

Practical Solutions

Confirm whether the cell type requires tissue-culture-treated plastic or a specific extracellular matrix coating.

Evaluate cell viability and follow cell-type-specific attachment and passaging recommendations.

5. Unexpected Changes in Cell Morphology

The Problem

Cells become rounded, elongated, enlarged, granular, or otherwise different from their usual appearance.

Possible Causes

- Cellular stress.
- Nutrient depletion.
- Excessive confluence.
- Contamination.
- Changes in differentiation state.
- Genetic drift or prolonged passaging.

Practical Solutions

Compare the cells with reference images from a validated source, examine culture conditions, and review passage history.

For established cell lines, periodically confirm identity using an appropriate authentication method.

Quick Troubleshooting Reference

Problem	Likely Causes	Recommended Checks
Slow growth	Medium, serum, incubation conditions	Verify medium and cell-line requirements
Low viability	Handling stress, toxicity	Review passaging and treatment conditions
Contamination	Microorganisms or contaminated reagents	Perform appropriate contamination testing
Poor attachment	Surface or coating incompatibility	Check vessel treatment and coating
Morphology changes	Stress, contamination, genetic drift	Review culture history and identity

Conclusion

Successful cell culture depends on suitable media, carefully controlled environmental conditions, good aseptic technique, and consistent cell handling.

Systematic evaluation of growth rate, viability, morphology, and contamination status can help researchers identify problems before they compromise experimental results.

Relevant Laboratory Products

EMDA International supplies cell culture media, sera, supplements, culture vessels, filtration products, and other laboratory consumables.

Email Now: sales@emdainternational.com

For research use only. Follow validated cell-line-specific protocols and laboratory biosafety procedures.