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5 CELL CULTURE CRISES and How to Fix Them!

When your cell culture goes awry, it can affect confidence in your results during every aspect of your downstream assays. Here, we discuss five common problems in a cell culture laboratory, and provide our top tips on how to fix them.

MYCOPLASMA AND OTHER BACTERIAL CONTAMINATION

ISSUE

Ubiquitous in the lab and often not visible under a microscope. Contaminants are routinely introduced into the incubator by scientists themselves as well as via contaminated laboratory equipment.

SOLUTION

Eradication through antibiotic treatment or discarding infected cell line. Perform regular monitoring - rapid mycoplasma and bacteria detection kits are a must! Use clean PPE, use proper aseptic technique, obtain cells from reliable source.

HUMAN ERROR

ISSUE

Incorrect handling is one of the most common issues in cell culture. Often, with routine comes carelessness. Outdated cell culture knowledge is passed down to new students.

SOLUTION

Take the time to treat your cells right! Ensure all students, postdocs, and lab technicians are trained using correct technique. Keep up-to-date with current best practices.

SUBOPTIMAL CULTURING AND SUBCULTURING CONDITIONS

ISSUE

Poor handling of cells results in subculture problems and uneven cell growth. Over-passaging (more than 10 passages or for more than 3 months). Passaging at the wrong time of the cell culture's growth cycle.

SOLUTION

Passage cells late in the log phase of growth, but before the stationary phase. At 70-95 % confluency to avoid reduced growth in subsequent subcultures. Use pipettes, pipette controllers, and cell analysis systems that won't let you down!

THAWING TRAUMA

ISSUE

Incorrect cell thawing results in non-viable cells. Slow thawing, thawing at the incorrect temperatures, immediately centrifuging post thawing at high rpms, and transferring to cold culture media can all cause cell stress.

SOLUTION

Thaw quickly! Transfer your tubes of frozen cells to a 37° C water bath immediately after removing from liquid nitrogen. Either don't centrifuge your cells immediately, or centrifuge at low rpm. Pre-warm a large volume of cell growth media before transferring your freshly thawed cells.

OVER-TRYPSINIZATION

ISSUE

Trypsin cleaves cell surface proteins when cells are exposed for too long or to a high concentration. Being overzealous with trypsin can lead to cell lysis. Loss of viability can also be an issue.

SOLUTION

Use low concentrations of trypsin. Monitor cells closely after trypsin addition with a microscope. Block and wash out trypsin.

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