Troubleshooting: Cell Culture

There are several common problems encountered when culturing cells. Problems in primary cultures may have different causes than the same problem in established cell lines.

The table below addresses some of the common problems encountered when culturing cells, along with their possible causes and suggested solutions.

Problem	Possible Cause	Suggested Solution
Rapid pH shift in medium	Incorrect carbon dioxide (CO_2) tension	Increase or decrease percentage of CO_2 in the incubator based on concentration of sodium bicarbonate in medium. For sodium bicarbonate concentrations of 2.0 to 3.7 g/L, use CO_2 amounts of 5% to 10%, respectively.
		Switch to CO_2-Independent Medium.
	Overly tight caps on tissue culture flasks	Loosen caps one-quarter turn.
	Insufficient bicarbonate buffering	Add HEPES buffer to a final concentration of 10 to 25 mM.
	Incorrect salts in medium	Use an Earle's salts-based medium in a CO_2 environment and a Hanks' salts-based medium in atmospheric conditions.
	Bacterial, yeast, or fungal contamination	Discard culture and medium.
		Try to decontaminate culture. (See *Decontaminating Cultures with Antibiotics and Antimycotics*.)
Precipitate in medium, no change in pH	Residual phosphate left over from detergent washing, which may precipitate powdered medium components	Rinse glassware in deionized, distilled water several times, then sterilize.
	Frozen medium	Warm medium to 37°C and swirl to dissolve. If precipitate remains, discard medium.
Precipitate in medium, change in pH	Bacterial or fungal contamination	Discard medium.
		Try to decontaminate culture. (See *Decontaminating Cultures with Antibiotics and Antimycotics*.)
Cells not adhering to culture vessel	Overly trypsinized cells	Trypsinize for a shorter time, or use less trypsin. (See *Dissociation of Cells from Culture Vessels*.)
	Mycoplasma contamination	Segregate culture and test for mycoplasma infection. Clean hood and incubator. If culture is contaminated, discard.
	No attachment factors in medium	For serum-free formulations, be sure they contain attachment factors.
Decreased growth of culture	Change in medium or serum	Compare media formulations for differences in glucose, amino acids, and other components.
		Compare the old lot of serum with the new lot in a growth experiment.
		Increase initial cell inoculum.
		Adapt cells sequentially to new medium.