

# Troubleshooting: Using Trypsin Solutions

Problem	Possible Causes	Suggestions
Cells are difficult to detach	<ul style="list-style-type: none"> <li>• Trypsin concentration is too low</li> <li>• Age of monolayer</li> <li>• Serum is still present on the monolayer</li> </ul>	<p>Increase the concentration of trypsin and/or add EDTA.</p> <p>Increasing the volume of trypsin/trypsin EDTA solution and placing the flask in 37°C. Subculture as the cells approach confluence. As a culture reaches confluence and is not promptly subcultured, cells become densely packed, preventing the trypsin from reaching the cell-substrate junction.</p> <p>Repeat rinsing of monolayer to remove all traces of serum which is a trypsin inhibitor.</p>
Low viability	<ul style="list-style-type: none"> <li>• Trypsin concentration is too high</li> <li>• pH or osmolality problems with the trypsin solutions</li> <li>• Pipetting or centrifuging</li> </ul>	<p>Trypsin may harm cell membranes at increased concentrations or when left on the cells for long periods of time. Lower the concentration of trypsin in the solution or decrease the reaction time.</p> <p>Verify the pH, and osmolality of the trypsin or trypsin/EDTA solution.</p> <p>Gently pipette the cell suspension until no visible clumps are visible. If centrifugation is necessary, decrease the time or RPMI.</p>
Cells are in clumps not a single-cell suspension	<ul style="list-style-type: none"> <li>• Cell-to-cell junctions are very tight (caused by increased age of monolayers)</li> </ul>	<p>Gently triturate suspension to encourage a single-cell suspension. Subculture before the monolayer reaches 100% confluence. Remove the medium from the culture vessel(s) by aspiration, then wash the monolayer with Ca<sup>+</sup> and Mg<sup>2+</sup>-free salt solution.</p>
Cells won't reattach to flask	<ul style="list-style-type: none"> <li>• Trypsin still present in the media</li> <li>• Not enough serum or attachment factors in media; flasks not cell culture treated</li> <li>• Cell membrane damage</li> <li>• Chemically-induced Cytotoxicity</li> </ul>	<p>Add serum-containing media to the cells once detachment is complete. Serum contains trypsin inhibitors, which inactivate the enzyme. Isolating the cells for the media through centrifugation may be necessary to remove trypsin, and then resuspend the cell pellet with media. Addition of trypsin inhibitors is also an option.</p> <p>Attachment factors may not be present in some serum-free media formulations. Add attachment factors to the culture media. Culture flasks are available pre-treated or coated and this coating, such a collagen, promotes attachments to the flask surface.</p> <p>Extensive incubation with trypsin may damage the cell membrane, specifically proteins on the cell surface. Decrease the enzyme concentration, shorten exposure time, and/or pipette more gently.</p> <p>It is important to store media and cells growing in cell culture medium away from sources of fluorescent light. Certain components in culture media are light sensitive (e.g. riboflavin, HEPES, tryptophan). This sensitivity may result in the production of H<sub>2</sub>O<sub>2</sub> and free radicals which are toxic to cells. The toxic effects of improperly stored media slowly increase with time.</p>