| Catalog No. | 25-050<br>1X, Liquid<br>0.25% Trypsin<br>in HBSS | 25-051<br>1X, Liquid<br>0.05% Trypsin/<br>0.53 mM EDTA<br>in HBSS | 25-052<br>1X, Liquid<br>0.05% Trypsin/<br>0.53 mM EDTA<br>in HBSS | 25-053<br>1X, Liquid<br>0.25% Trypsin/<br>2.21 mM EDTA<br>in HBSS | 25-054<br>10X, Liquid<br>2.5% Trypsin<br>in HBSS |
|---|---|---|---|---|---|
| Components | mg/L | mg/L | mg/L | mg/L | mg/L |
| D-Glucose | 1000.00 | 1000.00 | 1000.00 | 1000.00 | 1000.00 |
| EDTA•4Na•4$H_2O$ | — | 240.00 | 240.00 | 1000.00 | — |
| KCl | 400.00 | 400.00 | 400.00 | 400.00 | 400.00 |
| $KH_2PO_4$ | 60.00 | 60.00 | 60.00 | 60.00 | 60.00 |
| NaCl | 8000.00 | 8000.00 | 8000.00 | 8000.00 | 8000.00 |
| $NaHCO_3$ | 350.00 | 350.00 | — | — | 350.00 |
| $Na_2HPO_4$ (anhydrous) | 47.70 | 47.70 | 47.70 | 47.70 | 47.50 |
| Phenol red, Na | 10.00 | 10.00 | 10.00 | 10.00 | — |
| Trypsin 1:250 | 2500.00 | 500.00 | 500.00 | 2500.00 | 25000.00 |
| Specifications | | | | | |
| pH | 7.2 - 8.0 | 7.2 - 8.0 | 7.2 - 8.0 | 7.2 - 8.0 | 5.4 ± 0.3 |
| Osmolality (mOsm) | 280 - 320 | 270 - 320 | 270 - 320 | 270 - 320 | 320 - 475 |

Mediatech, Inc.

# Troubleshooting: Using Trypsin Solutions

| Problem | Possible Causes | Suggestions |
|---|---|---|
| Cells are difficult to detach | Trypsin concentration is too low | Increase the concentration of trypsin and/or add some EDTA |
| | Age of monolayer | Increasing the volume of trypsin/trypsin EDTA solution and placing the flask in 37C may help. Subculture as the cells approach confluence to prevent this problem. As a culture reaches confluence and is not promptly subcultured, cells become densely packed, preventing the trypsin from reaching the cell-substrate junction. |
| | Serum is still present on the monolayer | Repeat rinsing of monolayer to remove all traces of serum (trypsin inhibitor). |
| Low viability | Trypsin concentration is too high | An enzyme, 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. |
| | pH or osmolality problems with the trypsin solutions | Verify the expiration date, pH, and osmolality of the trypsin or trypsin/EDTA solution, as well as the date thawed. |
| | Pipetting or centrifuging | Gently pipette the cell suspension until no visible clumps are apparent. Decrease the time or RPM if centrifugation is necessary. |
| Cells are in clumps, not a single-cell suspension | Cell-to-cell junctions are very tight (caused by increased age of monlayers) | Gently triturate (pipette up and down) suspension to encourage a single-cell suspension. (Subculture before the monolayer reaches 100% confluence). |
| Cells won't reattach to flask | Trypsin still present in the media | Be sure to add serum-containing media to the cells once detachment is complete. Serum contains trypsin-inhibitors, which inactivate the enzyme and stop it from working. Isolating the cells from 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. |
| | Not enough serum or attachment factors in media; flasks not cell culture treated | Attachment factors may not be present in some serum-free media formulations. Add attachment factors to the culture media. Note: culture flasks are available pre-treated or coated; this coating, such a collagen, promotes attachments to the flask surface. |
| | Cell membrane damage | 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. |