

Dissociation of Cell Monolayers Using Trypsin Solutions

Guidelines for Use

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Most cell cultures grow in a layer or sheet that is the thickness of a single cell and is attached to a substrate. When subculturing adherent cells, these intercellular and cell-to-substrate connections must be broken. Proteolytic enzymes, such as trypsin, break these bonds, creating a single-cell suspension from which new subcultures are split.

Trypsin is available in several formulations, including solutions with or without EDTA. EDTA is a chelator that helps bind calcium and magnesium, allowing trypsin to access the cell-to-cell and cell-to-substrate bonds. More aggressive dissociation solutions contain higher concentrations of trypsin and EDTA, but higher concentrations can increase the risk of enzymatic damage to the cells. Higher concentration trypsin solutions should be used on a test monolayer prior to use.

When working in serum-free conditions, trypsin must be removed from contact with cells by centrifugation or deactivated by using a soybean trypsin inhibitor. Alternatively, use a non-enzymatic dissociation solution, such as Corning® Cellstripper® (25-056-CI), which gently dislodges loosely adherent cells using a mixture of chelators and does not require deactivation with serum or removal via centrifugation.

The following instructions are applicable to many cell lines. Actual procedures and concentrations should be determined empirically with specific cell lines. Regularly monitor cell viability at the subculture level to determine the most suitable conditions and procedures.

Procedure

- Step 1:** Pre-warm the trypsin solution to 37°C or other temperatures as needed (see step 4).
- Step 2:** Remove and discard the culture medium from the culture vessel (flask, plate, etc.).
- Step 3:** Carefully rinse the cell sheet with the appropriate amount of a balanced salt solution or trypsin solution and discard. Remove all traces of serum, as it contains trypsin inhibitors.
 - The monolayer may be washed with either calcium- and magnesium-free balanced salt solution (21-021-CV, 21-022-CV, or 21-031-CV) or the trypsin solution itself.
 - Testing the effects of each on a particular cell line will help determine the appropriate wash solution to use.
 - For sensitive cells, washing with a balanced salt solution is suggested to avoid damage to the cells.
- Step 4:** Add the trypsin solution to the side of the vessel opposite the cells and gently swirl the vessel to cover the monolayer. Allow cells to incubate several minutes and monitor for dissociation; cells will begin to round up and become loose. For monolayers that are particularly difficult to detach, the flask may be placed in a 37°C incubator for a short time. Alternatively, for cells that are sensitive to trypsinization, one can use trypsin at 2°C to 8°C to slow its enzymatic activity. Timing may vary depending on the cell type, age of monolayer, and other factors. Cells usually dissociate within 5 to 15 minutes.
- Step 5:** After cells appear detached, add an appropriate amount of complete growth medium containing serum.
- Step 6:** For serum-free conditions, add a trypsin inhibitor to neutralize the action of the trypsin. Gently triturate to disperse cells into suspension; vigorous pipetting may cause cell damage. If cells are too difficult to disperse, a more aggressive dissociation solution may be needed.

Ordering Information

Cat. No.	Description	Unit Size	Qty/Pk
25-050-Cl	Trypsin, 0.25% Trypsin in HBSS without calcium and magnesium	100 mL	6
25-051-Cl	Trypsin, 0.05% Trypsin/0.53 mM EDTA in HBSS with sodium bicarbonate, without calcium and magnesium	100 mL	6
25-052-Cl	Trypsin, 0.05%/0.53 mM in HBSS without sodium bicarbonate, calcium, and magnesium	100 mL	6
25-053-Cl	Trypsin, 0.25% Trypsin/2.21 mM EDTA in HBSS without sodium bicarbonate, calcium, and magnesium	100 mL	6
25-054-Cl	Trypsin, 2.5% Trypsin in HBSS without calcium, magnesium, and phenol red	100 mL	6
21-021-CV	Hank's Balanced Salt Solution, with sodium bicarbonate, without calcium, magnesium	500 mL	6
21-021-CM	Hank's Balanced Salt Solution, without calcium and magnesium	1L	6
25-056-Cl	Corning® Cellstripper®, liquid	100 mL	6

For more specific information on claims, visit the Certificates page at www.corning.com/lifesciences.

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