

## Dissociation of Cell Monolayers Using Trypsin Solutions

Most cell cultures grow as a single cell thick layer or sheet 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, a chelator that helps bind calcium and magnesium, allowing trypsin access to the cell-cell and cell-substrate bonds. More aggressive dissociation solutions may contain higher concentrations of trypsin and EDTA, but may also increase the risk of enzymatic damage to the cells. Higher concentration trypsin solutions should be tested on a test monolayer prior to use.

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

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The following instructions are applicable to many cell lines. Actual procedures and concentrations should be determined through experience with individual cell lines. Regularly monitor cell viability at subculturing to determine most suitable conditions and procedures.

### Procedure

1. Pre-warm the trypsin solution to 37°C (or other temperatures as needed - see step #4).
2. Remove and discard the culture medium from the culture vessel (flask, plate, etc).
3. Carefully rinse the cell sheet with the appropriate amount of a balanced salt solution or the trypsin solution, and discard. Removing all traces of serum is imperative, as serum contains trypsin inhibitors.
  - *The monolayer may be washed with either calcium-, and magnesium-free balanced salt solution (21-021 or 21-031) 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 balance salt solution may be best to avoid damage to the cells.*
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 that is 2-8°C to slow its enzymatic activity. Timing may vary depending on the cell type, age of monolayer, and other factors. Cells usually dissociate within 5-15 minutes.
5. Once cells appear detached, add an appropriate amount of complete (containing serum) growth medium.
6. For serum-free conditions, add a trypsin inhibitor to neutralize the action of the trypsin. Gently triturate to disperse cells into suspension; too vigorous pipetting may cause cell damage. If cells are too difficult to disperse without causing damage to them, a more aggressive dissociation solution may be needed.