
Dissociation of Cells from Primary Tissue

A common method to obtain single cell suspensions from primary tissue is enzymatic disaggregation. Expose the cells to enzymes for a minimal amount of time to preserve maximum viability. The following procedures disaggregate whole tissue to obtain a high yield of viable cells (1).

Trypsin

1. After dissecting off unusable tissue, mince the remaining tissue into 3- to 4-mm pieces with a sterile scalpel or scissors. Wash the tissue pieces by resuspending in a balanced salt solution without calcium and magnesium. Allow the tissue pieces to settle, and remove the supernate. Repeat the wash 2 or 3 times.
2. Place the container with the tissue pieces on ice, and remove any remaining supernate. Add 0.25% trypsin in a balanced salt solution without calcium or magnesium (~1 ml of trypsin for every 100 mg of tissue).
3. Incubate at 4°C for 6 to 18 h to maximize penetration of the enzyme with little trypsin activity.
4. Decant and discard the trypsin from the tissue pieces. Incubate the tissue pieces with residual trypsin at 37°C for 20 to 30 min.
5. Add warm complete medium to the tissue pieces and gently disperse the tissue by pipetting. If using a serum-free medium, also add a [soybean trypsin inhibitor](#).
6. Filter the cell suspension through sterile stainless steel mesh (100 to 200 µm) to completely disperse any remaining tissue. Count and seed the cells for culture.

TrypLE™ Products

TrypLE™ products are formulated to allow direct substitution into your existing protocols. The following general procedure can be used to remove various cell lines from cultureware while maintaining cellular integrity. Optimal conditions and concentrations employed for individual systems should be determined empirically.

1. Decant media from flask. Rinse flask with 5 ml of [Dulbecco's Phosphate Buffered Saline \(D-PBS\) without calcium and without magnesium](#) (GIBCO® cat. number 14190). Decant D-PBS.
2. Add an appropriate volume (i.e. 2 ml in a 75 cm² flask) of prewarmed TrypLE™ to flask. Rock vessel to coat cell sheet completely.
3. Incubate at 37°C until cells have detached (observe at 5 minute intervals). Gently rap vessel to dislodge cells.
4. Dilute in 2 to 5 ml of cell culture growth media and transfer cell suspension to a 15 ml centrifuge tube.
5. Centrifuge for 5 to 10 minutes at 100 × g. Discard supernatant and suspend cell pellet with 2 to 5 ml of fresh growth medium.

6. Determine viable and total counts.
7. Seed flasks according to normal protocol.
8. Incubate and subculture according to normal protocol depending on your cell type.

Note: Use of soybean trypsin inhibitor is not recommended.

Collagenase

1. Mince tissue into 3- to 4-mm pieces with a sterile scalpel or scissors. Wash the tissue pieces several times with [Hanks' Balanced Salt Solution \(HBSS\)](#).
2. Add [collagenase](#) (50 to 200 units/ml in HBSS).
3. Incubate at 37°C for 4 to 18 h. Addition of 3 mM CaCl₂ increases the efficiency of dissociation.
4. Filter the cell suspension through a sterile stainless steel or nylon mesh to separate the dispersed cells and tissue fragments from the larger pieces. Fresh collagenase can be added to the fragments if further disaggregation is required.
5. Wash suspension several times by centrifugation in HBSS.
6. Resuspend the pellet in culture medium. Count and seed the cells for culture.

Dispase

1. Mince tissue into 3- to 4-mm pieces with a sterile scalpel or scissors. Wash the tissue pieces several times in a calcium and magnesium-free balanced salt solution.
2. Add [dispase](#) (0.6 to 2.4 units/ml in calcium and magnesium-free balanced salt solution).
3. Incubate at 37°C for 20 min to several hours.
4. Filter the cell suspension through a sterile stainless steel or nylon mesh to separate the dispersed cells and tissue fragments from the larger pieces. Fresh dispase can be added to the fragments if further disaggregation is required.
5. Wash suspension several times by centrifugation in the balanced salt solution.
6. Resuspend the pellet in culture medium. Count and seed the cells for culture.

Reference:

1. Freshney, R. (1987) *Culture of Animal Cells: A Manual of Basic Technique*, p. 117, Alan R. Liss, Inc., New York.

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Dissociation of Cells from Culture Vessels

The following is a general procedure to rapidly remove various cell lines from the substratum while maintaining cellular integrity. This procedure is not meant to be universally applicable for all cell lines. The optimal conditions and concentrations employed for individual systems should be determined empirically.

- Monitor cell viability at the time of subculturing.
 - Cell viability should be greater than 90%.
 - For serum-free media, it is advisable to decrease the amount of trypsin used.
1. Remove and discard spent cell culture medium.
 2. Wash cells using a balanced salt solution without calcium and magnesium or wash with EDTA (see table to determine correct wash solution). Add wash solution to the side of the flask opposite the cells. Rinse the cell sheet by rocking the flask for 1 to 2 min and discard wash solution.
 3. Add the dissociation solution of choice (see table) at 2 to 3 ml/25 cm² to the side of the flask opposite the cells. Be sure that the dissociation solution covers the cell sheet. Incubate the flasks at 37°C. Rock the flasks gently. Generally, cells are dissociated in 5 to 15 min. The time needed to dissociate cells will vary according to cell line. Monitor the process carefully to avoid cell damage. Flasks of cell lines that are difficult to remove from the substratum may be tapped to expedite removal.
 4. When the cells are completely detached, stand the flask in the upright position to allow the cells to drain to the bottom of the flask. Add complete medium to the flask. Disperse the cells by pipetting repeatedly over the surface of the monolayer. Count and subculture the cells.

Note: For serum-free media, add soybean trypsin inhibitor. Usually using 1:1 (v:v) of a 0.25 mg/ml trypsin inhibitor solution to trypsin will inhibit trypsin.

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Dissociation of Cells from Culture Vessels, continued

Culture Type	Wash Solution	Dissociation Solution	GIBCO® Products and Cat. Nos.
Most continuous cell lines Strongly adherent continuous cell lines	HBSS or PBS without calcium and magnesium	Trypsin 0.25% in balanced salt solution without calcium and magnesium	0.25% Trypsin (liquid) 15050-065 2.5% Trypsin (liquid) (10X) 15090-046 Trypsin Powder (1:250) 27250-018
Many cells at early passage	D-PBS	TrypLE™ Products	TrypLE™ Select (liquid) 12563-011 TrypLE™ Express (liquid) 12605-010
Continuous cell lines where integrity of cell surface proteins is important	HBSS or PBS without calcium and magnesium	Trypsin 0.05% in 0.53 mM EDTA TrypLE™ Products	0.05% Trypsin 0.53 mM EDTA (liquid) 25300-054 0.05% Trypsin 0.53 mM EDTA (lyophilized) 15305-014 0.5% Trypsin 5.3 mM EDTA (10X) (liquid) 15400-054 TrypLE™ Select (liquid) 12563-011 TrypLE™ Express (liquid) 12605-010
Weakly adherent epithelial cells Transformed fibroblasts Primary cells where integrity of cell surface proteins is required	HBSS or PBS without calcium and magnesium	EDTA, glycerol in sodium citrate TrypLE™ Products	Enzyme-Free Cell Dissociation Buffers Hanks' based 13150-016 PBS based 13151-014 TrypLE™ Select (liquid) 12563-011 TrypLE™ Express (liquid) 12605-010
Strongly adherent early-passage cell lines	HBSS or PBS without calcium and magnesium	Trypsin 0.25% in 1 mM EDTA Dispase 0.6 to 2.4 units/ml in PBS	0.25% Trypsin 1 mM EDTA liquid 25200-056 Trypsin Powder (1:250) 27250-018 Dispase 17105-041
Epithelial cells ^a	0.5 mM to 1 mM EDTA	0.5 mM to 1 mM EDTA Dispase 0.6 to 2.4 units/ml in PBS	Versene 1:5,000 (0.53 mM EDTA in PBS) 15040-066 Dispase 17105-041
Strongly adherent cells ^a Epithelial cells Some tumor cells	0.5 mM to 1 mM EDTA	Trypsin 0.25% with 1 mM EDTA Dispase 0.6 to 2.4 units/ml in PBS without calcium and magnesium	0.25% Trypsin 1 mM EDTA 25200-056 Versene 1:5,000 (0.53 mM EDTA in PBS) 15040-066 Trypsin Powder (1:250) 27250-018 Dispase 17105-041
Thick cultures, multiple layers ^a Dense cultures that are collagen rich	1 mM EDTA	Trypsin 0.25% Collagenase 200 units/ml, 1 mM EDTA in balanced salt solution without calcium and magnesium	0.25% Trypsin 1 mM EDTA 25200-056 Collagenase 17100, 17101, 17102, 17104 Trypsin Powder (1:250) 27250-018
All adherent cultures	HBSS or PBS without calcium and magnesium	Scrape off cell sheet ^b	—

^a Some cells may be sensitive to EDTA.

^b Scraping may cause mechanical damage and will not yield a single cell suspension.

Note: Adapted from Freshney, R. (1987) *Culture of Animal Cells: A Manual of Basic Technique*, p. 129, Alan R. Liss, Inc., New York.

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