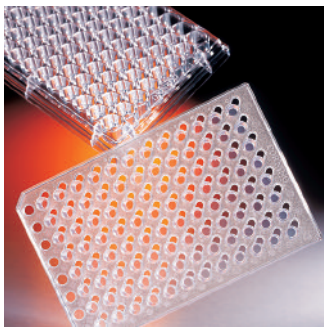
Trypsinization Procedure for Corning® Transwell® Inserts



Materials

- Calcium- and magnesium-free phosphate-buffered saline (PBS)
- Cell dissociation solution: such as 0.05% trypsin/EDTA
- Quenching solution: medium containing serum, trypsin inhibitor for serum-free culture, or PBS for non trypsin cell dissociation solutions.
- Appropriate collection containers for cells
- Phase contrast microscope

Procedure

Helpful Hint: When working with 12 and 24 well Transwell inserts, a multichannel pipettor can be used to transfer reagents by using every other tip.

1. Aspirate spent medium from the receiver (bottom chamber) well of the plate and then from the inside the Transwell insert. Be careful to avoid touching the membrane.
2. Rinse the top and then the bottom of the Transwell insert with PBS to remove any serum which contains trypsin inhibitors. See table for recommended buffer volumes.

   Note: For loosely adherent cells, one PBS rinse is sufficient. You may want to visually check the PBS for cells that may have detached before aspirating and add these cells to the collection vessel, if needed.

   For cells that are strongly adherent, we recommend two to three PBS rinses to fully remove serum. Also, allowing the cells to incubate in PBS for 10 to 15 minutes before aspirating may speed up the trypsinization of some strongly adherent cells. Be sure to check the PBS rinse for detached cells before aspirating.

Transwell	Insert Surface Area (cm^2)	Recommended Insert Volume (mL)	Recommended Receiver Well Volume (mL)
96 well HTS	0.143	0.05	0.100
24 well	0.33	0.1	0.3
12 well	1.12	0.25	0.6
6 well	4.67	0.6	1
75 mm insert	44	5	6

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3. Add trypsin/EDTA or other dissociation solution to the Transwell® insert and receiver well. Use the same liquid volumes as above. Incubate in a 37°C incubator containing 5% CO_2 until cells are rounded and floating. This time will vary, depending on how adherent and confluent the cell line is. Periodically monitor cell detachment under a microscope.

   Helpful Hint: If you are having difficulty visualizing cell detachment in your Transwell insert, try setting up a cell culture dish or flask at the same cells/cm^2 and use this to monitor cell detachment.

4. Once the cells look rounded and detached, add quenching solution to inserts and receiver wells to inactivate the dissociation solution. Gently pipet up and down to break up cell clumps.

   Note: If not all cells are detached, tapping the Transwell plate against a hard surface should dislodge any remaining cells. Be careful not to cause cell damage or to splash liquid on the lid of the plate.

5. Collect cell suspension from inserts and receiver wells and transfer to a suitable storage container.

6. Rinse the top and bottom of the Transwell insert with PBS (see table on front) to remove any remaining cells. Add this solution to the cell suspension.

7. Observe Transwell inserts under a phase contrast microscope to ensure that all of the cells have been removed. Perform a second PBS rinse or trypsinization if there are large numbers of cells still remaining on the membrane.

   Note: If cell suspension is clumpy try gently pipetting solution up and down several times until a single cell suspension is attained.

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