

Preparation of 21-Day Caco-2 Cell Monolayers for BD Falcon™ 96-Multiwell Insert Systems

Protocol

Purpose

To describe the procedure for preparation of 21-day Caco-2 cell monolayers on BD Falcon™ 96-Multiwell Inserts, 1.0 µm membrane pore size.

Handling Tip: Move plates slowly with an even motion when transferring into and out of an incubator. DO NOT TILT plate during handling.

Materials

• Inserts:

– BD Falcon 96-Multiwell Insert System [1.0 µm, 0.0804 cm² membrane surface area] (Cat. Nos. 351130 and 351131 includes feeder tray, Cat. No. 353938 includes receiver plate)

• Receiver Plates:

– BD Falcon 96-Square Well, Angled-Bottom Plates (Cat. No. 353925)

• Standard cell culture facility, including laminar flow hood, humidified, 37°C/5% CO₂ incubators, inverted microscope

• BD Falcon tissue culture flasks, serological pipets, and 50 ml centrifuge tubes

• Dulbecco's Modified Eagle Medium (DMEM) High Glucose (Life Technologies Cat. No. 11965-092 or equivalent)

• Fetal Bovine Serum (FBS) (Life Technologies Cat. No. 26140-079 or equivalent)

• Phosphate Buffered Saline (PBS) without calcium and magnesium

• Trypsin-EDTA solution (Invitrogen Cat No. 25300-062 or equivalent)

• Caco-2 Cells (ATCC Cat. No. HTB-37)

Procedure

Cell Culture (stocks in BD Falcon tissue culture flasks)

1. Caco-2 cell cultures are routinely maintained in BD Falcon 175 cm² tissue culture (TC) flasks (Cat. No. 353112) in DMEM medium containing 10% FBS (DMEM/FBS). The cells are split to 2.5 x 10⁶/flask every 7 days with refeeding every 48-72 hours.
2. Stock cell cultures are examined with an inverted microscope for typical healthy cell morphology and adequate density just prior to trypsinization for monolayer set-up.

Monolayer Preparation and Maintenance

1. Remove culture medium from flask and wash monolayer 1x using PBS without calcium and magnesium.
2. Add 5 ml of trypsin solution and incubate at 37°C until cells lift from plastic. This generally takes 5-7 minutes.
3. Meanwhile, add 25-30 ml of DMEM/FBS medium to the feeder tray, and store in the incubator until needed. Alternatively, the 96-well receiver plate (Cat. No. 353925) can be used to culture the inserts (add 260 µl of culture medium to each well).
4. Add 20 ml of DMEM/FBS medium to inactivate the trypsin and triturate the cells 10 times.
5. Transfer to a 50 ml tube.
6. Take a 0.5 ml sample for cell counting, and spin the cells down at 1,000 rpm for 5-7 minutes at room temperature. Resuspend cells at approximately 1.48 x 10⁵ cell/ml in DMEM/FBS medium. To determine the optimal seeding density for your cell type on a porous growth surface, we recommend using a range of seeding densities (cells/cm²) that brackets the seeding density used on nonporous surfaces (flasks, dishes, and plates). For example, if you currently seed at 1.0 x 10⁵ cells/cm², seed at 0.5 x 10⁵, 1.0 x 10⁵, and 5.0 x 10⁵ cells/cm² to determine the optimal seeding density. (The membrane surface area is 0.08 cm², therefore, to seed 1.04 x 10⁴ cells to each apical chamber, add 70 µl of the cell suspension.)
7. Place an insert plate into the feeder tray or 96-well receiver plate prepared above and add 70 µl of the cell suspension to each apical chamber of the insert.
8. Transfer system to the incubator.
9. Replace apical and basolateral DMEM/FBS medium every 48-72 hours. Maintain an apical volume of 70 µl throughout the culturing period.

Note: The following volumes are recommended:

Culture period of monolayers/inserts:

- 70 µl culture medium in the apical chambers
- 25-30 ml culture medium in the feeder tray, or 260 µl culture medium in the receiver plate wells

Permeability/Transport Assay:

- 50-70 µl transport buffer in the apical chambers
- 260 µl transport buffer in the receiver plate wells