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

Protocol

CORNING

Purpose

To describe the procedure for preparation of 21-day Caco-2 cell monolayers on 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:
  - Falcon 96 Multiwell Insert System [1.0 µm, 0.0804 cm^2 membrane surface area] (Cat. Nos. 351130 and 351131 includes feeder tray, Cat. No. 353938 includes receiver plate)
- Receiver Plates:
  - Falcon 96 Square Well, Angled-Bottom Plates (Cat. No. 353925)
- Standard cell culture facility, including laminar flow hood, humidified, 37°C/5% CO_2 incubators, inverted microscope
- 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 (Life Technologies Cat. No. 25300-062 or equivalent)
- Caco-2 Cells (ATCC Cat. No. HTB-37)

Procedure

Cell Culture (stocks in Falcon tissue culture flasks)

1. Caco-2 cell cultures are routinely maintained in Falcon 175 cm^2 tissue culture (TC) flasks (Cat. No. 353112) in DMEM medium containing 10% FBS (DMEM/FBS). The cells are split to 2.5 x 10^6/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 10^5 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/cm^2) that brackets the seeding density used on nonporous surfaces (flasks, dishes, and plates). For example, if you currently seed at 1.0 x 10^5 cells/cm^2, seed at 0.5 x 10^5, 1.0 x 10^5, and 5.0 x 10^5 cells/cm^2 to determine the optimal seeding density. (The membrane surface area is 0.08 cm^2, therefore, to seed 1.04 x 10^4 cells to each apical chamber, add 70 µL of the cell suspension.)
7. Place a 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

Corning acquired the Falcon® brand.
For additional Corning product, technical, or distributor information, please e-mail us at CLSTechServ@corning.com, visit our website www.corning.com/lifesciences or call 800.492.1110. Outside the United States, call 978.442.2200.
For information on the acquisition, visit www.corning.com/discoverylabware.

Corning Incorporated
Life Sciences
836 North St.
Building 300, Suite 3401
Tewksbury, MA 01876
t 800.492.1110
t 978.442.2200
f 978.442.2476

www.corning.com/lifesciences

Worldwide Support Offices

ASIA/PACIFIC

Australia/New Zealand
t 0402-794-347

China
t 86 21 2215 2888
f 86 21 6215 2988

India
t 91 124 4604000
f 91 124 4604099

Japan
t 81 3-3586 1996
f 81 3-3586 1291

Korea
t 82 2-796-9500
f 82 2-796-9300

Singapore
t 65 6733-6511
f 65 6861-2913

Taiwan
t 886 2-2716-0338
f 886 2-2516-7500

EUROPE

France
t 0800 916 882
f 0800 918 636

Germany
t 0800 101 1153
f 0800 101 2427

The Netherlands
t 31 20 655 79 28
f 31 20 659 76 73

United Kingdom
t 0800 376 8660
f 0800 279 1117

All Other European Countries
t 31 (0) 20 659 60 51
f 31 (0) 20 659 76 73

LATIN AMERICA

Brasil
t (55-11) 3089-7419
f (55-11) 3167-0700

Mexico
t (52-81) 8158-8400
f (52-81) 8313-8589

CORNING | FALCON® cellgro® AXYGEN® PYREX® GOSSELIN™

For Research Use Only. Not for use in diagnostic or therapeutic procedures.
For a listing of trademarks, visit us at www.corning.com/lifesciences/trademarks.
Corning Incorporated, One Riverfront Plaza, Corning, NY 14831-0001