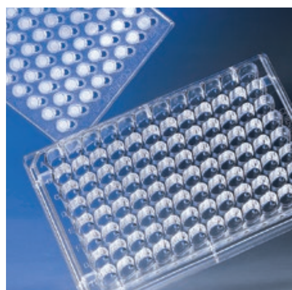
Transwell®-96 Permeable Supports for Drug Transport

Guidelines for Use

CORNING



Introduction

These protocols are being provided as a starting point for drug transport assays such as those utilizing the Caco-2 or MDCK cell lines. It is not intended to represent an optimized protocol for your laboratory. Corning is dedicated to assisting you in obtaining the best possible results for your assays; however, it is the end user's responsibility to optimize and validate methods to insure robust results. Factors that should be optimized include, but are not exclusive of: media type and components, frequency of feeding cultures, handling and manipulating the plates, method for removal of and replacement of aqueous phase media and buffers, cell line, passage number, selection and storage, sterile techniques, and incubation times. If assistance is required, please refer to the protocol below, or contact your local Corning representative.

Preparation of Cells for Cell Seeding

1. Grow cells to desired confluency in the appropriate vessel (i.e., flask or plate).
2. Aspirate media, and rinse one time with PBS.
3. Add 0.05% trypsin/EDTA solution and allow cell monolayer to detach.
4. Inactivate trypsin/EDTA by adding excess serum containing medium.
5. Remove cell suspension to a conical tube and pellet cells by centrifugation at 250 x g for 5 minutes.
6. Resuspend cells in seeding medium to achieve a density between 100,000 and 200,000 c/cm^2.

Seeding of Cells into Transwell Plates

1. Add 75 to 100 µL of above cell suspension to each insert of a Transwell plate.
2. Add 25 mL medium to reservoir
3. Place plate at 37°C, 5% CO_2.

REMINDER: Handle plate carefully to avoid excessive media movement in the reservoir.

Feeding and Media Changes

NOTE: A series of access ports (arrows) adjacent to column 1 have been designed into the Transwell insert to enable aspiration of the reservoir without removing the Transwell insert.

NOTE: The sequence of media removal and replacement is critical to success of any assay that requires the cells to adhere to the membrane surface. When feeding the Transwell inserts it is important to maintain net positive hydrostatic pressure above the cells to prevent cells from being pushed off the membrane. Therefore, whether growing in a receiver or a reservoir plate, in manual mode or with automation, the sequence of media removal/addition is always the same:

1. Aspirate media from the lower chamber (receiver or reservoir plate).
2. Aspirate media from the wells in the upper chamber (apical access).
3. Add media to the upper chamber wells (apical access).
4. Add media to the lower chamber (receiver or reservoir plate).

Procedure for Full Medium Change

1. Remove plate(s) from incubator and place in hood.
2. Aspirate medium from reservoir through the access ports along the side column 1.

   NOTE: A 2 mL aspirating pipet (Corning Cat. No. 9016) can easily accomplish this.
3. Aspirate medium from each Transwell® insert with the 8-channel aspiration manifold.

   NOTE: For removal of medium, Corning recommends using an 8-channel manifold (Corning Cat. No. 3389, or Cadence Science Cat. No. 6113).

   NOTE: A small amount of medium will be retained over the cell monolayer.
4. Add 100 µL of growth medium to each well of the Transwell insert(s).
5. Add 20 to 25 mL of growth medium to the reservoir tray(s).

   NOTE: 25 mL of growth media is the maximum recommended fluid volume in the reservoir and is added if all the medium is removed in step 2.
6. Return plate(s) to the incubator.

   NOTE: The frequency of medium changes will depend on the cell type and culture period. If your medium contains a pH indicator, use its color as a guide for when the medium should be changed. For 21 or 28 day Caco-2 cultures, it is recommended to change medium every three days for the first two weeks, followed by alternating days for the remainder of the culture. For MDCK cells, which typically culture for 4 to 5 days, change the medium the day before the assay.

Assessment of Cell Monolayer Integrity

There are many ways to evaluate the integrity of cell (i.e., Caco-2) monolayers on membrane supports. The two most common are Transepithelial Electric Resistance (TEER) and Lucifer Yellow (LY) rejection. All manipulations must be performed using aseptic technique for cells that will remain in culture. Procedures can be carried out non-aseptically on a lab bench for cells that are at the end of their culture period.

TEER Measurement

TEER measures the resistance to pass current across a cell monolayer. Corning recommends using a World Precision Instruments electrode and an Epithelial Tissue Volt/Ohm Meter for this procedure.

1. Add 235 µL of room temperature growth medium to each well of a receiver plate.
2. Remove plate(s) to be tested from incubator and allow to come to room temperature.
3. Place Transwell insert to be tested into the receiver plate prepared in step 1.
4. Place TEER meter probe into each well insert [short probe in Transwell (apical side), the long probe into the receiver well (basolateral side)], and record reading (ohms).
5. Once all wells have been measured, return the plate to the incubator or continue processing the plate (stain cell monolayer, Lucifer yellow transport assay, or drug transport assay).

   Calculation of TEER values:

   TEER measurement (ohms) × Area of membrane (cm^2) = TEER value (ohm • cm^2)
   i.e., 2,320 x 0.143 = 331.76 ohm • cm^2

Lucifer Yellow Rejection

Lucifer Yellow (LY) travels across cell (i.e., Caco-2) monolayers only through passive paracellular diffusion (through spaces between cells) and has low permeability. Therefore, it is not able to pass across cell monolayers when tight junctions between cells are maintained. Permeability (Papp) for LY of ≤5 to 12 nm/s has been reported to be indicative of well-established Caco-2 monolayers. For quantification of LY transport it is important to use standard curves (step 9) and to use black plates for optimal sensitivity.

1. Pre-warm all reagents before beginning assay:
   a. HBSS (Hank's Balanced Salt Solution) containing Ca++ and Mg++
   b. HBSS 1% DMSO (310 µL total needed for each well to be analyzed, including standards).
2. Aliquot 235 µL of HBSS 1% DMSO to each well of a new receiver plate and place in the incubator.

3. Remove Transwell® plate from incubator.
4. Wash each Transwell insert 3 times with pre-warmed HBSS (no DMSO) following steps for Feeding of Transwell inserts.

   NOTE: HBSS should also be changed in the receiver or reservoir plate to efficiently wash the monolayers.

   NOTE: For final wash, insert can be held vertically, and virtually all medium can be removed.
5. Add 75 µL of 60 µM LY solution (in HBSS 1% DMSO) to each well of the Transwell insert.
6. Remove prepared receiver plate from the incubator.
7. Place the Transwell insert into receiver plate of Step 2.
8. Incubate the plate at 37°C, 5% CO_2 for 1 hour while shaking (50 rpm).
9. During incubation make dilutions for standard curves:

   NOTE: Make LY solutions in HBSS 1% DMSO.

   NOTE: We recommend starting with 60 µM and making 1:3 serial dilutions for 20 µM and 6.7 µM as a high standard curve. For a low standard curve we recommend starting at 1 µM and doing a series of 1:3 dilutions to 4.1 nM.
10. Aliquot standards in triplicate to a solid black plate (Corning Cat. No. 3915).

    NOTE: We recommend keeping the high standards and the low standards spatially removed from one another as high standards can affect the reading of nearby low standards. Also include 3 wells of HBSS 1% DMSO as a blank.
11. Transfer 100 µL from each well of the receiver plate into the corresponding well of a solid black plate.
12. Transfer 50 µL from each well of a Transwell insert into the corresponding well of another solid black plate containing 50 µL of HBSS 1% DMSO.
13. Read plates in appropriate fluorescent reader.
    (Excitation/Emission wavelength 480 (20) nm/530 (20) nm).
14. Calculate LY rejection or Pc values.

Calculation of Results

Permeability coefficient (Pc)

Receiver volume in mL (V_r)

Membrane growth area in cm^2 (A)

Initial apical concentration µM(C_I)

Final receiver concentration µM(C_f)

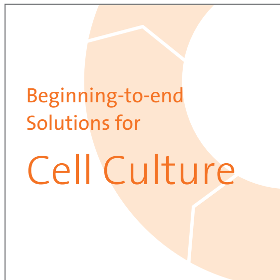
Assay time in seconds (t)

$$Pc = (V_r \times C_f)/C_I \times A \times t$$

Or

$$\% \text{ LY rejection} = 100\,[1 - RFU_{basolateral}/RFU_{apical}]$$

REMINDER: Value for initial concentration obtained from the standard curve must be multiplied by two to obtain correct concentration, as the samples are diluted 1:2 for reading. See step 12 under Lucifer Yellow.



www.corning.com/lifesciences/solutions

At Corning, cells are in our culture. In our continuous efforts to improve efficiencies and develop new tools and technologies for life science researchers, we have scientists working in Corning R&D labs across the globe, doing what you do every day. From seeding starter cultures to expanding cells for assays, our technical experts understand your challenges and your increased need for more reliable cells and cellular material.

It is this expertise, plus a 160-year history of Corning innovation and manufacturing excellence, that puts us in a unique position to offer a beginning-to-end portfolio of high-quality, reliable cell culture consumables.

For more specific information on claims, visit the Certificates page at **www.corning.com/lifesciences**.

Warranty/Disclaimer: Unless otherwise specified, all products are for research use only. Not intended for use in diagnostic or therapeutic procedures. Corning Life Sciences makes no claims regarding the performance of these products for clinical or diagnostic applications.

For additional product or technical information, visit **www.corning.com/lifesciences** or call 1.800.492.1110. Outside the United States, call +1.978.442.2200 or contact your local Corning sales office.

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 61 427286832

China
t 86 21 3338 4338
f 86 21 3338 4300

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 6572-9740
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 020 655 79 28
f 020 659 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
grupoLA@corning.com

Brasil
t (55-11) 3089-7400

Mexico
t (52-81) 8158-8400

CORNING | FALCON AXYGEN GOSSELIN PYREX

For a listing of trademarks, visit www.corning.com/clstrademarks.
All other trademarks are the property of their respective owners.

© 2009, 2017 Corning Incorporated. All rights reserved. Printed in USA 1/17 CLS-AN-058-REV2