

Lucifer Yellow Permeability Assay using Falcon® HTS 96 Multiwell Insert Systems

CORNING

Protocol

For permeability studies, Caco-2 cell monolayers grown on Falcon HTS 96 Multiwell Insert Systems (Cat. Nos. 351130 or 351131) should be placed onto a Falcon 96 square well, angled-bottom plate (Cat. No. 353925) for analysis of lucifer yellow permeability. Although the lid and feeder tray of the Falcon HTS 96 Multiwell Insert System is non-directional, the insert plate is designed to be placed on the Falcon 96 square well, angled-bottom plate (Cat. No. 353925) in one unique orientation to prevent cross contamination of wells. To properly align the Falcon 96 Multiwell Insert Plate in the Falcon 96 square well, angled-bottom plate, make sure the Falcon logos on the top of both pieces face the same direction. The sampling ports on the insert plates face the same direction as the notched corner side of the 96 square well, angled-bottom plate.

Note: The standard Falcon 96 well plates are not compatible with the Falcon 96 Multiwell Insert System. Use of standard 96 well plates will result in media wicking up on the sides of the wells and possibly into the insert or out of the well.

Materials

- ▶ Caco-2 cells grown on Falcon HTS 96 Multiwell Insert System, 1.0 µm pore size (Cat. Nos. 351130 or 351131)
- ▶ Falcon 96 square well, angled-bottom plate and lid (Cat. No. 353925)
- ▶ Lucifer Yellow (Molecular Probes)
- ▶ Transport buffer (HBSS with Ca²⁺, Mg²⁺, +10 mM HEPES, pH 7.4, phenol-red free)
- ▶ Platform orbital shaker
- ▶ Fluorescence plate reader

Lucifer Yellow Permeability Assay

Transport buffer (HBSS with Ca²⁺, Mg²⁺, +10 mM HEPES, pH 7.4) is added to the basal compartment. Lucifer yellow is diluted in transport buffer and added to the apical compartment at a final concentration of 100 µM. The monolayers are placed on a shaker at 70-90 rpm in a 37°C incubator with 90% relative humidity and 5% CO₂ for 1-2 hours. Fluorescence leakage was determined for lucifer yellow by 485 nm excitation and 530 nm emission using a fluorescence plate reader.

- Remove the Falcon 96 Multiwell Insert Plate from its feeder tray and place it directly on the Falcon 96 square well, angled-bottom plate (Cat. No. 353925, sold separately).
- Gently remove medium from each insert. Wash gently with transport buffer.
- Gently add 50 µL of lucifer yellow dissolved in transport buffer (100 µM) to the inside of each insert.
- Add 250-275 µL of transport buffer to each well of the Falcon 96 square well, angled-bottom plate.
- Incubate in a 37°C incubator (5% CO₂ and 90% humidity) for 1-2 hours on an orbital shaker set for 70-90 rpm.
- For a lucifer yellow standard curve, add increasing concentrations of lucifer yellow solution ranging from 0.1-50 µM to a separate Falcon 96 square well, angled-bottom plate.

- g) Following incubation, remove the Falcon 96 Multiwell Insert Plate from the 96 square well, angled-bottom plate and set aside. Lucifer yellow fluorescence in the 96 square well, angled-bottom plate (fluorescence leakage across the Caco-2 monolayer) is read directly in a fluorescence plate reader using a 485 nm excitation and an emission filter of 530 nm. The standard curve plate is also read directly in the fluorescence plate reader.
- h) Use the standard curve to calculate the lucifer yellow concentration in each well. These values can then be used to determine the % flux and permeability coefficients.

Permeability Measurements

The apical-to-basal permeability coefficients (P_c) can be calculated according to the following equation:

$P_c = (V/(A \times C_i)) \times (C_f/T)$ where V is the volume of the basal chamber (mL), A is the area of the membrane insert (cm²), C_i is the initial concentration of the drug μM or fluorescence units/ml added, C_f is the final concentration of the drug (μM or fluorescence units/ml), and T is the assay time (seconds). Typical volumes include 50 μL in apical compartment and 270 μL transport buffer in basal compartment. Area of membrane = 0.0804 cm².

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®** **GOSELIN™**

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