

Lucifer Yellow Permeability Assay Using BD Falcon™ HTS 96-Multiwell Insert Systems

Protocol

For permeability studies, Caco-2 cell monolayers grown on the BD Falcon™ HTS 96-Multiwell Insert Systems (Cat. Nos. 351130 or 351131) should be placed onto a BD Falcon 96-square well, angled-bottom plate (Cat. No. 353925) for analysis of lucifer yellow permeability. Although the Lid and Feeder Tray of the BD Falcon HTS 96-Multiwell Insert Systems are non-directional, the insert plate is designed to be placed on the BD Falcon 96-square well, angled-bottom plate (Cat. No. 353925) in one unique orientation to prevent cross contamination of wells. To properly align the 96-Multiwell Insert Plate in the 96-square well, angled-bottom plate, make sure the BD 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 BD Falcon 96-well plates are not compatible with the 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 BD Falcon HTS 96-Multiwell Insert System, 1 mm pore size
- BD 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
- Fluorescent 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 BD Falcon 96-Multiwell Insert Plate from its Feeder Tray and place it directly on the BD 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 BD 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 BD Falcon 96-square well, angled-bottom plate.
- Following incubation, remove the BD 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 fluorescent plate reader using a 485 nm excitation and an emission filter of 530 nm. The standard curve plate is also read directly in the fluorescent plate reader.
- 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

Yes, donor information is provided on the batch data sheet that is shipped with the product. The donor information includes donor gender, age, race, cause of death, social history, medical history, and medications taken while in the hospital. 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.