Corning® BioCoat™ Pre-coated PAMPA Plate System

Catalog Number 353015

Guidelines for Use

Discovery Labware, Inc., Two Oak Park, Bedford, MA 01730, Tel: 1.978.442.2200 (U.S.)
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For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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Introduction

Drug compounds are screened for their oral absorption potential early in the drug discovery process in order to eliminate poor performers and to identify candidates that need to be modified. Parallel artificial membrane permeability assays (PAMPA) have become a very useful tool for predicting in vivo drug permeability and are well-suited as a ranking tool for the assessment of compounds with passive transport mechanisms. Use of the PAMPA assay allows for ranking of compounds into a low or high classification by using UV VIS spectroscopy or LC/MS.

The Corning BioCoat Pre-Coated PAMPA Plate System is a 96-well insert system with a PVDF filter plate which has been pre-coated with structured layers of phospholipids and a matched receiver microplate. The Corning BioCoat Pre-Coated PAMPA Plate System has been validated for use in PAMPA and comes ready to use in your assay.

Each pair of filter plate and receiver plate is individually packaged in a foil bag. **Store the package at –20°C immediately upon receipt.**

Recommended Procedure for Permeability Assay

Step 1: Prior to use, the pre-coated PAMPA plate system should be warmed to room temperature for at least 30 minutes. While the plate system is warming, the compound solutions can be prepared by diluting stock solutions into a buffer (e.g. PBS).

Note: Once a pre-coated PAMPA plate is warmed to room temperature, it must be used within 24 hours.

Step 2: Add 300 µL of compound solutions (e.g. 100 – 200 µM in PBS) per well in the **receiver plate (donor plate)**.

Step 3: Add 200 µL of buffer (e.g. PBS) per well in the **filter plate (acceptor plate)**.

Step 4: Place the filter plate on the receiver plate by slowly lowering the pre-coated PAMPA plate until it sits on the receiver plate. Incubate the assembly at room temp for 5 hours.

Step 5: Separate the pre-coated PAMPA plate and the receiver plate. Determine the compound concentrations in both plates using UV VIS spectroscopy or LC/MS. Calculate the permeability of each compound using the formula listed in the next section.

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Recommended Formula for Data Analysis

Permeability (in unit of cm/s):

$$P_e = \frac{-\ln[1 - C_A(t)/C_{equilibrium}]}{A * (1/V_D + 1/V_A) * t}$$

Mass Retention:

$$R = 1 - [C_D(t) * V_D + C_A(t) * V_A]/(C_0 * V_D)$$

Where:

C_0 = initial compound concentration in donor well (mM)

C_D (t) = compound concentration in donor well at time t. (mM)

C_A (t) = compound concentration in acceptor well at time t. (mM)

V_D = donor well volume = 0.3 mL

V_A = acceptor well volume = 0.2 mL

$C_{equilibrium} = [C_D(t) * V_D + C_A(t) * V_A]/(V_D + V_A)$

A = filter area = 0.3 cm^2

t = incubation time = 18000 s (= 5 hr)

Example:

If the initial concentration and final concentrations (at 5 hr) of a compound are measure to be

C_0 = 200 μM

C_D (t) = 180 μM

C_A (t) = 10 μM

Then the mass retention and permeability of this compound are

$$C_{equilibrium} = [180 * 0.3 + 10 * 0.2]/(0.3 + 0.2) = 112 \mu M$$

$$P_e = \frac{-\ln[1 - 10/112]}{0.3 * (1/0.3 + 1/0.2) * 18000} = 2.078 * 10^{-6} cm/s$$

$$R = 1 - [180 * 0.3 + 10 * 0.2]/(200 * 0.3) = 0.067 = 6.7\%$$

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Note: The permeability equation is deduced from the two-flux equation:
(Avdeef *et al.*, *Eur. J. Phar. Sci.* 2001, 14, 271-280)

$$C_A(t) = M/(V_D+V_A) + (C_A(0) - M/(V_D+V_A))\exp\{-P_eA(1/V_D+1/V_A)t\}$$

M refers to the total amount (mol) of the drug in the system. When there is mass retention, M is replaced by the total amount minus the amount of sample lost.

Please visit www.corning.com/lifesciences to download spreadsheets that contain the formula for calculating permeability.

Product Specifications

Design and Materials: • Microplate Type • Filter plate • Filter plate PAMPA coating • Filter plate lid • Receiver microplate	 • 96 well microplate configuration, complies with ANSI standards • Polystyrene with 0.4 µm PVDF membrane • Phospholipids • Polystyrene • Polystyrene
Pre-coated filter plate compatibility:	• Verified compatibility with 5% DMSO and 20% methanol
Automation compatibility:	• Suitable for use with most liquid handling equipment
Corning® BioCoat™ Pre-coated PAMPA Plate system QC:	• PAMPA assay performed using a set of standard compounds

SAFETY RECOMMENDATION: Handle in accordance with good industrial hygiene and laboratory safety practices.

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Revision History				
Rev.	**Change No.**	**Description of Change**	**Revised By**	**Revised Date**
1	ECO60481	Initial issue	Kevin Chen	2/8/07
2	CC 02516	Updated to Corning branding and prot variable fields and add in safety statement	L. Brown	07/28/2014
3	CC-18815	Re-brand as Corning BioCoat	Salihe	11/6/20

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