Parallel Artificial Membrane Permeability Assay-Skin Kit (PMSKN-096)
Quantitative Determination of Skin Membrane Permeability

DESCRIPTION

MEMBRANE PERMEABILITY is an important characteristic to determine for evaluating compounds as potential drug candidates. Drugs often need to cross cell membranes in order to reach their target of action and this makes a compound’s ability to passively cross these membranes an important characteristic to evaluate. The skin, in particular, the stratum corneum is a complex barrier, which can be mimicked. Rapid and early screening of compounds for skin penetration is highly desirable for drug discovery. Permeability can be evaluated by cell-based methods; however, these methods are often expensive and time consuming. Parallel Artificial Permeability Assays (PAMPA) offer researchers a quick, inexpensive method of evaluating the permeability of test compounds. Our PMSKN-096 kit is designed to aid in evaluating skin permeability.

BioAssay Systems’ PMSKN Kit provides all the necessary components to run a Parallel Artificial Permeability Assay for skin permeability studies.

KEY FEATURES

Convenient: Includes all necessary equipment to run a PAMPA plate.

Simple and low-cost: Procedure is easy to follow and more affordable than cell-based permeability assays.

High-throughput: Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

APPLICATIONS

Direct Assays: Assess membrane permeability of test compounds.

KIT CONTENTS (96 TESTS)

Donor Plate: 1 Plate

Acceptor Plate: 1 Plate

Skin Mimic Solution: 2.0 mL

High Permeability Medium Permeability Control: 120 µL

Low Permeability Medium Permeability Control: 120 µL

Storage conditions: The kit is shipped at room temperature. Store Permeability Controls and Skin Mimic Solution at -20°C upon receiving; store Donor Plate and Acceptor Plate at room temperature. Shelf life: 12 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

Reagent Preparation: Equilibrate all components to room temperature prior assay. Briefly centrifuge tubes before opening.

PROCEDURES

Prepare Skin Mimic Solution: Bring the Skin Mimic Solution to room temperature and ensure that it is completely dissolved and homogeneous. The solution comes ready to use.

Prepare Test Compound Stock Solutions: Prepare 10 mM stock solutions in DMSO for all compounds being assayed. The supplied Permeability Controls are provided as 10 mM solutions in DMSO.

Assay Procedure using 96-well plate

1. In separate centrifuge tubes, prepare 500 µL of 500 µM Test Compound: mix 25 µL 10 mM Test Compound in DMSO + 475 µL PBS. If using the Permeability Controls, dilute them to 500 µM as well: mix 25 µL Permeability Control + 475 µL PBS.

2. In separate tubes, prepare 200 µM Equilibrium Standards for each test compound and control: mix 80 µL of 500 µM Test Compound or Control with 120 µL PBS. If the compound is able to permeabilize the membrane and fully reach equilibrium, 200 µM will be the final concentration of solution in the Donor and Acceptor wells. Next, in a separate tube, mix 5 µL DMSO + 245 µL PBS to prepare the Blank Control. Set aside the Equilibrium Standards and Blank Control for analysis the next day.

3. Add 300 µL PBS to wells in the acceptor plate.

4. With the donor plate still in its tray, add 17 µL of Skin Mimic solution directly to the well membranes of the donor plate. Be careful not to puncture the membranes with the pipette tip.

5. Add 200 µL of each 500 µM Test Compound and 500 µM Permeability Controls to duplicate wells of the donor plate.

6. Carefully place the donor plate into the acceptor plate wells. Incubate at RT or 37°C for 18 hours or the desired incubation time period (e.g. 16 – 24 hours).

7. Carefully remove donor plate and collect the liquid in acceptor plate wells for analysis. This will be referred to as Accepto Plate Solution.

8. Add 100 µL of Acceptor Solution and Equilibrium Standards for each Test Compound and Permeability Control. Also add 100 µL Blank Control to wells of UV plate (Cat # P96UV).

9. Read Absorbance spectrum from 200nm to 500nm in 10nm intervals to determine peak absorbance of test compounds. The Blank Control is to confirm peaks are due to the test compound and not the DMSO in the solution. Peak absorbance for High Permeability, Medium Permeability, and Low Permeability Controls are 260nm, 260nm, and 270nm respectively.

NOTE: Alternatively, analysis can be done using HPLC, MS, or other methods of quantification.

DATA ANALYSIS

Using the determined peak absorbance for each respective test compound and Permeability Control, determine the Permeability Rate (Pₑ) using the following calculation:

\[ P_e = \frac{C \times \ln(1 - \frac{OD_A}{OD_E})}{OD_A} \text{ cm/s} \]

Where ODₐ is the absorbance of Acceptor Solution minus Blank, ODₑ is the absorbance of the Equilibrium Standard minus Blank, and, using an 18 hour incubation, \( C = 7.72 \times 10^3 \). If a different incubation time than 18 hours was used, please adjust C accordingly using the equation below.

\[ C = \frac{V_D \times V_A}{(V_D + V_A) \times Area \times time} \text{ cm/s} \]

In this protocol, Donor Volume \((V_D) = 0.2 \text{ cm}^3\), Acceptor Volume \((V_A) = 0.3 \text{ cm}^3\), Membrane Area (Area) = 0.24cm², and time is 64,800 s (18 hr x 3600 s/hr = 64,800 s).

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, DMSO, PBS, UV Plates (Cat # P96UV), and an absorbance plate reader capable of absorbance spectrums.
Permeability Controls
Permeability Controls PAMPA using PBS, 17 µl Skin Mimic Solution, and 18 hour incubation at 25°C.

LITERATURE