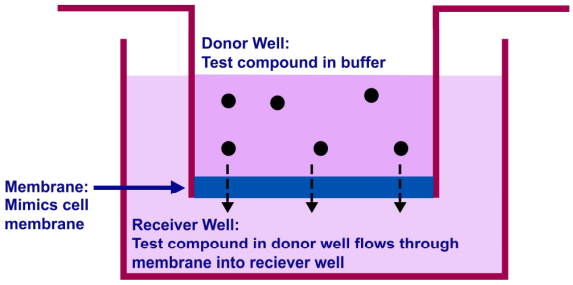
Parallel Artificial Membrane Permeability Assay-BBB Kit (PMBBB-096)

Quantitative Determination of Blood Brain Barrier (BBB) 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 Blood Brain Barrier (BBB) is made of brain endothelial cells with tight junctions. Rapid and early screening of compounds for BBB 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 PMBBB-096 kit is designed to aid in evaluating BBB permeability.

BioAssay Systems' PMBBB Kit provides all the necessary components to run a Parallel Artificial Permeability Assay for Blood Brain Barrier studies.



Parallel Artificial Permeability Assay
Principle of PAMPA Assay method.

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 BBB membrane permeability of test compounds.

KIT CONTENTS (96 TESTS)

Donor Plate: 1 Plate	Dodecane: 1.5 mL
Acceptor Plate: 1 Plate	Dried Brain Lipids: 1 Tube
High Permeability Control: 120 µL	Medium Permeability Control: 120 µL
Low Permeability Control: 120 µL	

Storage conditions: The kit is shipped at room temperature. Store Permeability Controls, Dodecane, and Dried BBB Lipids 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 BBB Solution: Prepare BBB Lipid Solution in dodecane by resuspending the dried brain lipids with 600 µL dodecane. Pipette up and down repeatedly (~100 times) until all dried brain lipids have been solubilized; vortexing or sonication can assist with this. Once solubilized, the lipids should be stored at -20°C and used within 6 days.

Prepare Test Compound Stock Solutions: Prepare 10 mM stock solutions in DMSO for all test compounds. The supplied Permeability Controls are provided as 10mM 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 equibilibrium, 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 5 µL of BBB Lipid Solution in Dodecane 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.

   Note: we recommend running all experimental variables in at least duplicate.
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 Acceptor 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 250nm, 250nm, 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 (P_e) using the following calculation:

$$P_e = C \times -\ln\left(1 - \frac{OD_A}{OD_E}\right) \text{ cm/s}$$

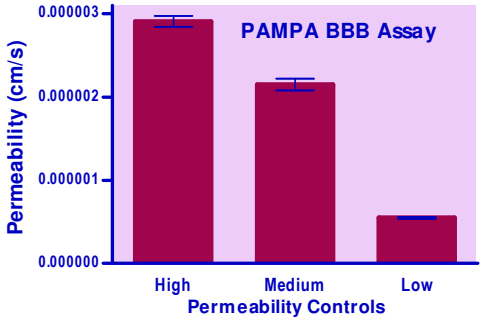
Where OD_A is the absorbance of Acceptor Solution minus Blank, OD_E is the absorbance of the Equilibrium Standard minus Blank, and, using an 18 hour incubation, $C = 7.72 \times 10^{-6}$. If a different incubation time than 18 hours was used, please adjust C accordingly using the equation below.

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

In this protocol, Donor Volume (V_D) is 0.2 cm^3, Acceptor Volume (V_A) is 0.3 cm^3, Membrane Area (Area) is 0.24cm^2, and time is 64,800 s (18 hr × 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 PAMPA using PBS, BBB membrane, and 18 hour incubation at 25°C.

LITERATURE

1. Wohnsland, F., Faller, B. (2001). High-throughput Permeability pH Profile and High-throughput Alkane/Water Log P With Artificial Membranes. J Med Chem 44: 923-930.
2. Kansy, M., et al (1998). Physiochemical High Throughput Screening: Parallel Artificial Membrane Permeation Assay in the Description of Passive Absorption Processes. J Med Chem 41: 1007-1010.
3. Di, Li., et al (2002). High throughput artificial membrane permeability assay for blood—brain barrier. J Med Chem 38: 223-232.