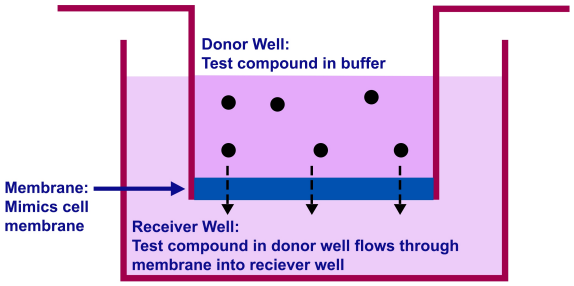
Parallel Artificial Membrane Permeability Assay Kit (PAMPA-096)

Quantitative Determination of 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. 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.

BioAssay Systems' PAMPA Kit provides all the necessary components to run a Parallel Artificial Permeability Assay.



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 membrane permeability of test compounds.

KIT CONTENTS (96 TESTS)

Donor Plate:	1 Plate	**Dodecane:**	2 mL
Acceptor Plate	1 Plate	**Dried Lecithin:**	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 Lecithin 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 Lecithin Solution: Prepare 4% lecithin in dodecane solution by resuspending the dried lecithin with 750 µL dodecane. Pipette up and down repeatedly (~ 100 times) until all dried lecithin has been solubilized; vortexing or sonication can assist with this. If you would like to run the PAMPA at a lower concentration of lecithin, you may use the chart below to further dilute the lecithin to your desired percentage. *Alternatively, you can use your own selection of lipid mixture. The lipid solution and concentration should be selected to best mimic the target barrier to be evaluated.*

4% Lecithin + Dodecane	% Lecithin
600 µL + 0 µL	4
450 µL + 150 µL	3
300 µL + 300 µL	2
150 µL + 450 µL	1

Prepare Test Compound Stock Solutions: Prepare 10mM stock solutions in DMSO for all compounds being assayed. 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 10mM 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 4% Lecithin 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
5. 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)
6. Carefully remove donor plate and collect the liquid in acceptor plate wells for analysis. This will be referred to as Acceptor Solution
7. 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).
8. 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 280nm, 270nm, 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:

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

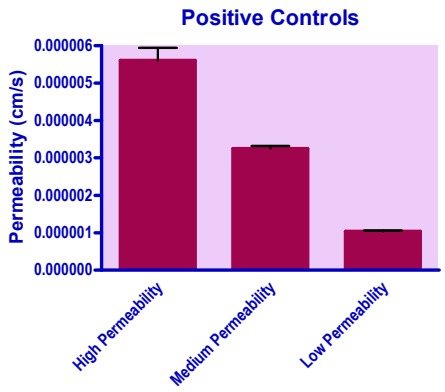
Where OD_A is the absorbance of Acceptor Solution, OD_E is the absorbance of the Equilibrium Standard, and, using an 18 hour incubation, $C = 7.72\times10^{-6}$. If a different incubation time than 18 hours was used, please adjust C accordingly using the equation below.

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

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

Permeability Controls PAMPA using PBS, 4% lecithin 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.