TECHNICAL MANUAL

Parallel Artificial Membrane Permeability Assay (PAMPA) Kit

- **SKU CODE:** BA0259
- **SIZE:** 96T
- **RUO:** Research-Use-Only

Revised: 06/2026

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Please read entire manual carefully before starting experiment.

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1. 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.

2. Storage & Expiry

The expiration date is indicated on the outer label of the kit box.

The kit is shipped at room temperature. Store Permeability Controls, Dodecane, and Dried Lecithin at -20°C upon receiving; store Donor Plate, Acceptor Plate, and Working Tray at room temperature. Shelf life: 12 months after receipt.

3. Kit Contents

No	Component Name	Specifications
1	Donor Plate	1 Plate
2	Acceptor Plate	1 Plate
3	Working Tray	1 Plate
4	Dodecane	1.5 mL
5	Dried Lecithin	1 Tube
6	High Permeability Control	120 µL
7	Medium Permeability Control	120 µL
8	Low Permeability Control	120 µL

Note: *All reagents must be stored according to the specified conditions listed in the table above. Do not mix reagents from different kits, as this may compromise assay*

performance. For reagents provided in small volumes, centrifuge briefly before use to ensure complete recovery of the contents.

4. Product 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.

Assay Genie's Parallel Artificial Membrane Permeability Assay (PAMPA) Kit provides all the necessary components to run a Parallel Artificial Permeability Assay for gastrointestinal (GI) membrane permeability.

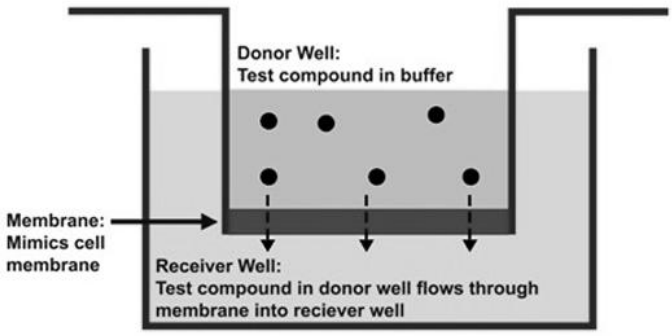

Figure 1: Parallel Artificial Permeability Assay - Principle of the PAMPA assay method. A test compound in the donor well diffuses across an artificial membrane (mimicking a cell membrane) into the receiver well.

5. Applications

Direct Assays: Assess membrane permeability of test compounds.

6. Important Notes

1. This assay kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
2. Please read the instructions carefully before beginning the assay. Ensure that all instruments are correctly calibrated. Strict adherence to the protocol is essential for accurate results.
3. Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves.
4. If the concentration of the target substance falls outside the detection range, please adjust the sample by performing further dilution or concentration as needed.
5. If your sample type is not listed in the instruction manual, we strongly recommend performing a preliminary test to confirm compatibility.
6. Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.

7. Assay Procedure

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

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 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.

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 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 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.*

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 280nm, 270nm, and 270nm respectively.

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

8. Calculation

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

$$\mathbf{Pe = C \times -ln(1 - OD_A / OD_E)\ cm/s}$$

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 = 7.72×10^{-6}. If a different incubation time than 18 hours was used, please adjust C accordingly using the equation below.

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

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).

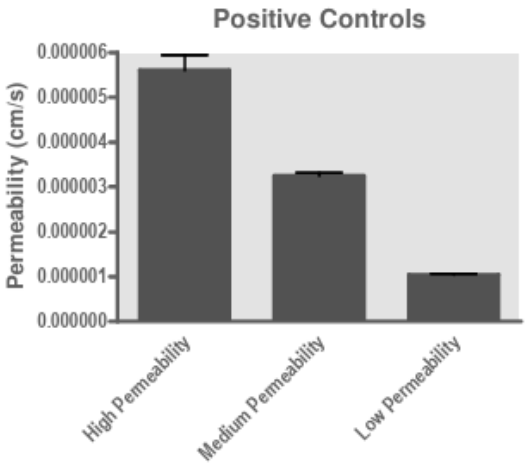

Figure 2: Permeability Controls — bar chart of measured permeability (cm/s) for the High, Medium, and Low Permeability Controls, run using PBS, a 4% lecithin membrane, and an 18-hour incubation at 25°C. Permeability decreases progressively from the High to the Low Permeability Control.

9. Materials Required (But Not Provided)

Pipetting devices, DMSO, PBS, UV Plates, and an absorbance plate reader capable of absorbance spectrums.

Notes:

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.