Parallel Artificial Membrane Permeability Assay-Skin Kit (BA0262)

Product Datasheet | SKU BA0262

Description

PAMPA kit providing all components required to run a 96-well artificial membrane permeability assay for the quantitative determination of skin membrane permeability.

Specifications

Detection Method	Parallel Artificial Membrane Permeability Assay (PAMPA); UV absorbance (200-500 nm)
Sample Type	Test compounds
Species Reactivity	All
Assay Time	18 hours incubation (typically 16-24 hours)
Kit Size	96 Assays
Equipment Required	Microplate reader (UV/absorbance spectrum capable)
Storage	Store Permeability Controls and Skin Mimic Solution at -20°C; store Donor Plate, Acceptor Plate and Working Tray at room temperature
Shelf Life	12 months
Shipping	Room Temperature

Kit Components

Component	Quantity	Storage
Donor Plate	1 Plate	Room temperature
Acceptor Plate	1 Plate	Room temperature
Working Tray	1 Plate	Room temperature
Skin Mimic Solution	2.0 mL	-20°C
High Permeability Control	120 µL	-20°C
Medium Permeability Control	120 µL	-20°C
Low Permeability Control	120 µL	-20°C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0262	96 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. Equilibrate all components to room temperature and briefly centrifuge tubes before opening. Bring the Skin Mimic Solution to room temperature and ensure it is completely dissolved and homogeneous (it comes ready to use).
2. Prepare 10 mM test compound stock solutions in DMSO (the supplied Permeability Controls are provided as 10 mM DMSO solutions).
3. In separate tubes, prepare 500 µL of 500 µM Test Compound (25 µL 10 mM compound + 475 µL PBS); dilute Permeability Controls to 500 µM similarly.
4. Prepare 200 µM Equilibrium Standards for each compound and control (80 µL of 500 µM + 120 µL PBS), and a Blank Control (5 µL DMSO + 245 µL PBS); set aside for next-day analysis.
5. Add 300 µL PBS to wells in the acceptor plate.

6. With the donor plate still in its tray, add 17 µL Skin Mimic Solution directly to the well membranes, taking care not to puncture them.
7. Add 200 µL of each 500 µM Test Compound and Permeability Control to duplicate wells of the donor plate (run all variables at least in duplicate).
8. Carefully place the donor plate into the acceptor plate wells and incubate at RT or 37°C for 18 hours (or 16-24 hours as desired).
9. Carefully remove the donor plate and collect the liquid in the acceptor plate wells (Acceptor Solution).
10. Add 100 µL of Acceptor Solution, Equilibrium Standards and Blank Control to wells of a UV plate (Cat # P96UV).
11. Read the absorbance spectrum from 200 nm to 500 nm in 10 nm intervals to determine peak absorbance; peak absorbance for High, Medium and Low Permeability Controls are 260 nm, 260 nm and 270 nm respectively. Alternatively, analysis can be performed by HPLC, MS or other quantification methods.

Example Permeability Control Values (PMSKN-096)

Compound	Permeability (cm/s)
Chloramphenicol	1.98 x 10-7
Promazine HCl	1.40 x 10-6
Methyl Paraben (High)	1.51 x 10-5
Diclofenac	2.52 x 10-6
Clonidine	1.91 x 10-6
Ethyl Paraben	1.23 x 10-5
Theophylline (Low)	2.23 x 10-7
Propyl Paraben (Medium)	6.44 x 10-6

Calculation

Determine the Permeability Rate (Pe) using Pe = C × -ln(1 - ODA/ODE) cm/s, where ODA is the absorbance of the Acceptor Solution minus Blank and ODE is the absorbance of the Equilibrium Standard minus Blank. For an 18-hour incubation, C = 7.72×10^-6. For other incubation times, calculate C = (VD × VA) / ((VD + VA) × Area × time) cm/s, where Donor Volume (VD) = 0.2 cm3, Acceptor Volume (VA) = 0.3 cm3, Membrane Area = 0.24 cm2, and time = 64,800 s for 18 hours.

Notes

- Run all experimental variables in at least duplicate.
- The Blank Control confirms that peaks are due to the test compound and not the DMSO in solution.
- Analysis may alternatively be performed using HPLC, MS or other methods of quantification.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.