Parallel Artificial Membrane Permeability Assay-BBB Kit (BA0261)

Product Datasheet | SKU BA0261

Description

PAMPA kit providing all components required to run a 96-well artificial membrane permeability assay for the quantitative determination of blood-brain barrier 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, Dodecane and Dried BBB Lipids 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
Dodecane	1.5 mL	-20°C
Dried Brain Lipids	1 Tube	-20°C
High Permeability Control	120 µL	-20°C
Low Permeability Control	120 µL	-20°C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0261	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. Prepare BBB Lipid Solution by resuspending the dried brain lipids in 600 µL dodecane, pipetting up and down (~100 times) until fully solubilised; store at -20°C and use within 6 days.
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 pH 7.2); 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 pH 7.2), and a Blank Control (5 µL DMSO + 245 µL PBS pH 7.2); set aside for next-day analysis.

5. Add 300 µL PBS (pH 7.2) to wells in the acceptor plate.
6. With the donor plate still in its tray, add 5 µL BBB Lipid Solution in dodecane 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 the High and Low Permeability Controls are 250 nm and 270 nm respectively.

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.
- Store BBB Lipid Solution at -20°C and use within 6 days of preparation.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.