EA204 – ExpressPlus P-gp Substrate Assessment in MDR1-MDCK Cells (+/- Inhibitor)

This assay is used to determine the interaction of a test article with P-gp using MDR1-MDCK cell monolayers with and without a P-gp inhibitor

Catalog number EA204

Required from Sponsor

- Either a minimum of 300 µL of test article at 10 mM in DMSO, or 5 mg of powder
- Molecular mass (exact mass) of the test article and its salt form
- MSDS or handling and storage information e.g., light sensitive, store at -20°C, etc.

Deliverables

- Cell batch QC results
- The percent recovery of the test article from the assay wells containing MDR1-MDCK monolayers
- The apparent permeability ($P_{\text{app}}$) of the test article in both directions in the presence and absence of inhibitor
- The efflux ratio in the presence and absence of inhibitor
- The brain penetration potential of a test article classified as either low, moderate, or high using the following criteria:
  - Low: $P_{\text{app}} A\rightarrow B < 3.0 \times 10^{-6}$ cm/s
  - Low: $P_{\text{app}} A\rightarrow B \geq 3.0 \times 10^{-6}$ cm/s, Efflux $\geq 10$
  - Moderate: $P_{\text{app}} A\rightarrow B \geq 3.0 \times 10^{-6}$ cm/s, $10 >$ Efflux $\geq 3.0$
  - High: $P_{\text{app}} A\rightarrow B \geq 3.0 \times 10^{-6}$ cm/s, Efflux $< 3.0$
- P-gp substrate classification:
  - Positive: Efflux ratio $\geq 2.0$ in the absence of inhibitor and corrected efflux ratio reduced by $\geq 50\%$ in the presence of inhibitor
  - Negative: Efflux ratio $\geq 2.0$ in the absence of inhibitor and corrected efflux ratio reduced by $< 50\%$ in the presence of inhibitor
  - Negative: Efflux ratio $< 2.0$ in both the absence and presence of inhibitor

Substrate

- Test article at 5 µM in HBSSg with maximum DMSO concentration $\leq 0.8\%$

Assay System

- Confluent monolayers of MDR1-MDCK cells, 7 to 12 days old

Assay Conditions
- Bidirectional permeability of the test article in MDR1-MDCK cells in the presence and absence of inhibitor
- Transport buffer: HBSSg, pH 7.4 ± 0.2
- Receiver well containing 1% BSA
- Apical and basolateral side at pH 7.4
- Dose two cell monolayers (n=2), in the presence and absence of inhibitor
  - Dose apical side for (A→B) assessment
  - Dose basolateral side for (B→A) assessment
- Sample from both apical and basolateral sides at 120 minutes
- Determine the concentrations of test article using a generic LC-MS/MS method with a minimum 6-point calibration curve

**Assay QC**

- The quality of the monolayer batch is verified using control compounds before the monolayers are released for use
- The quality of each monolayer used in the assay is verified by calculating the $P_{app}$ for the control article, lucifer yellow, dosed post-experimentally

**Notes**

1. The results from this assay are sent to the sponsor in the *Express*Plus report format, which may include graphical representations of data and comparison with historical data for reference articles.
2. This screening assay does not provide definitive information about the P-gp article interactions or the apparent $K_m$.
3. Assay conditions with the inhibitor include:
   - A 30 minute pre-incubation with Valspodar
4. Assay conditions without the inhibitor include:
   - A 30 minute pre-incubation with buffer
5. Digoxin can be run as an optional positive control
   - Dosed at 10 µM
   - BSA is not included in the receiver chamber