



Mechanistic Permeability Studies in the Caco-2 Cell Tissue Culture Model

This non-GLP assay is used to mechanistically investigate the absorption characteristics of a test article by measuring its permeability under various conditions in the Caco-2 tissue culture model.

- Required from Customer**
- A minimum 100 mg of drug
 - Molecular mass (exact mass) of the test compound and its salt form
 - MSDS or handling and storage information, e.g., light sensitive, store at $-20\text{ }^{\circ}\text{C}$, and other compound information (solubility, etc.)
 - Highest human dose strength (if known)

- Deliverables**
- Apparent permeability (P_{app}) of test compound across Caco-2 cell monolayers from the apical to basolateral (A \rightarrow B) or basolateral to apical (B \rightarrow A) side up to eight different conditions relative to metoprolol (high permeability standard):

<input type="checkbox"/> pH 5.5 (A \rightarrow B)	<input type="checkbox"/> Conc 1 (A \rightarrow B)	<input type="checkbox"/> Conc 1 (B \rightarrow A)	<input type="checkbox"/> Other.....
<input type="checkbox"/> pH 6.5 (A \rightarrow B)	<input type="checkbox"/> Conc 2 (A \rightarrow B)	<input type="checkbox"/> Conc 3 (B \rightarrow A)	<input type="checkbox"/> Other.....
<input type="checkbox"/> pH 7.4 (A \rightarrow B)	<input type="checkbox"/> Conc 3 (A \rightarrow B)	<input type="checkbox"/> Pgp inh. (A \rightarrow B)	<input type="checkbox"/> Other.....

- Mass balance of test article for assessment of binding to tissue and/or transwells
 - Preliminary BCS permeability classification (High permeability: $P_{eff\text{ test}}/P_{eff\text{ metoprolol}} > 1$)
 - Efflux ratio ($P_{app\text{ B}\rightarrow\text{A}}/P_{app\text{ A}\rightarrow\text{B}}$): an efflux ratio ≥ 3.0 is classified as significant
- Substrate**
- Test compound in MES or HEPES buffer together with the high permeability marker metoprolol with $< 1\%$ DMSO
- Assay System**
- Confluent monolayers of Caco-2 cells 18 to 27 days old
 - The integrity of the monolayers will be assessed by measuring the Transepithelial Electrical Resistance (TEER)
- Assay Conditions**
- Receiver well contains modified Hanks buffer HBSS (MES or HEPES buffered)
 - Three monolayers dosed in each direction ($n = 3$)
 - Apply substrate solution to apical side for (A \rightarrow B) assessment and to basolateral side for (B \rightarrow A) assessment
 - Sample both apical and basolateral sides over 2 hrs in 15 min intervals
 - At the end of the study, the integrity of the monolayer will be assessed by measuring the transport of Lucifer Yellow, a non-absorbable fluorescence marker
 - All samples will be assayed by HPLC or LC-MS/MS with a minimum of a four point calibration curve and acceptance criteria of $r^2 \geq 0.90$ and $\%RE \leq 20\%$ ($\leq 30\%$ for lowest standard). Lucifer Yellow samples will be read on a fluorescence plate reader
- Data Analysis**
- Effective permeability (P_{eff}) is calculated following published methods¹
- Quality Control**
- QC review of raw and processed data.

¹ P. Artursson* and J. Karlsson: Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial cells. BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, Vol. 175, No. 3, 1991(880-885)