

Permeability Screening in the Caco-2 Cell Tissue Culture Model

This non-GLP assay is used to provide an initial estimate of the absorption potential of a test article by measuring its permeability in the Caco-2 tissue culture model.

Required from Customer	<ul style="list-style-type: none"> • A minimum of either 300 μL of test compound at 10 mM in DMSO, or 5 mg of powder • 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°C, and other compound information (solubility, etc.)
Deliverables	<ul style="list-style-type: none"> • Mass balance of test article for assessment of binding to tissue and/or transwells • Apparent permeability (Papp) of test compound across Caco-2 cell monolayers from the apical to basolateral (A\rightarrowB) and the basolateral to the apical (B\rightarrowA) side at one pH and one concentration • Preliminary BCS permeability classification (High permeability: $\text{Peff}_{\text{test}}/\text{Peff}_{\text{metoprolol}} > 1$) • Efflux ratio (Papp B\rightarrowA)/(Papp A\rightarrowB): an efflux ratio ≥ 3.0 is classified as significant
Substrate	<ul style="list-style-type: none"> • Test compound in MES buffer pH 6.5 at 10 μM together with the high permeability marker metoprolol with $< 1\%$ DMSO
Assay System	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • Receiver well contains modified Hanks buffer HBSS (MES or HEPES buffered) • Apply substrate solution to apical side for (A\rightarrowB) assessment and to basolateral side for (B\rightarrowA) assessment • Sample initial substrate solution and both apical and basolateral sides at 15 and 60 min • 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 relative to the original dosing solution (response only). Lucifer Yellow samples will be read on a fluorescence plate reader
Data Analysis	<ul style="list-style-type: none"> • Effective permeability (Peff) is calculated following published methods¹
Quality Control	<ul style="list-style-type: none"> • 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)