



Permeability Screening in the MDCK Cell Tissue Culture Model

This non-GLP assay is used to provide an estimate of the absorption characteristics of a test article by measuring its permeability in the MDCK 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 (P_{app}) of test compound across MDCK 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: $P_{eff\text{ test}}/P_{eff\text{ metoprolol}} > 1$)• 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	<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 MDCK cells 4 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)• Two monolayers dosed in each direction ($n = 3$)• 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 (P_{eff}) 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)