



Preliminary BCS Permeability Classification in the Rat *In Situ* Perfusion Model

This non-GLP assay is used to determine a preliminary BCS permeability classification by measuring the permeability of a test compound at three concentrations that bracket the anticipated dosing range in the rat *in situ* perfused jejunum.

Required from Customer	<ul style="list-style-type: none">• Highest human dose strength• Minimum 50 mg of test compound in powder form• Any available solubility and stability data of test compound• Molecular mass (exact mass) of test compound and its salt form• MSDS or handling and storage information, e.g., store at $-20\text{ }^{\circ}\text{C}$, light-sensitive, etc.
Deliverables	<ul style="list-style-type: none">• Stability of test compound in MES buffer and intestinal perfusate.• Effective jejunal permeability (P_{eff}) at three concentrations of test compound in the rat <i>in situ</i> perfusion model relative to the high permeability standard metoprolol• Preliminary BCS permeability classification (High permeability: $P_{\text{eff}}_{\text{test}}/P_{\text{eff}}_{\text{metoprolol}} > 1$)• Preliminary assessment of concentration dependent permeability and potential for efflux
Substrate	<ul style="list-style-type: none">• Test compound at three concentrations (highest human dose dissolved in 250 mL, and 10% and 1% of the high dose concentration) in MES buffer pH 6.5 ± 0.2
Assay System	<ul style="list-style-type: none">• Perfusion studies will be conducted in male albino Sprague-Dawley rats, 9-10 weeks old and weighing 250-400 g, fasted for 18 h with free access to water• Rats are anesthetized either with an im injection of ketamine/xylazine and butorphanol or by 2% isoflurane• A jejunal segment of approximately 10 cm is cannulated on two ends.
Assay Conditions	<ul style="list-style-type: none">• Perfusion solution containing the drug in 10 mM MES buffer, pH 6.5, 135 mM NaCl, 5 mM KCl, 0.01% ^{14}C-PEG 4000, metoprolol (high permeability marker) is passed through the jejunal segment at a flow rate of 0.2 mL/min• After steady state is reached, samples are taken in 10 min intervals for 1 h• All samples including perfusion samples at different time points and original drug solution will be assayed by HPLC or LC-MS/MS with a minimum of a four point calibration curve and by scintillation counting
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 Jae-Seung Kim, Stefanie Mitchell, Paul Kijek, Yasuhiro Tsume, John Hilfinger, and Gordon L. Amidon: The Suitability of an *In Situ* Perfusion Model for Permeability Determinations: Utility for BCS Class I Biowaiver Requests. Molecular Pharmaceutics VOL.3, NO.6: 686-694