



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

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

Required from Customer	<ul style="list-style-type: none">• A study design defining all study aspects documented in a sponsor approved study protocol• Highest human dose strength• Minimum 1g of test compound in powder form• Solubility 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°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• Definitive BCS permeability classification (High permeability: $P_{\text{eff}_{\text{test}}}/P_{\text{eff}_{\text{metoprolol}}} > 1$)• Assessment of concentration dependent permeability and potential for efflux• Study report including QA statement and GLP compliance statement
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">• Prior to study conduct, stability of the test article will be determined in MES buffer and intestinal perfusate• Perfusion studies will be conducted in male albino Sprague-Dawley rats, approximately 9-10 weeks old and weighing 250-400 g, fasted overnight with free access to water• Rats are anesthetized either with an i.m. injection of ketamine/xylazine and butorphanol or by 2% isoflurane• A jejunal segment of approximately 10 cm is cannulated on two ends
Assay Condition	<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) and a low permeability marker is passed through the jejunal segment at a flow rate of 0.1-0.2 mL/min• After steady state is reached, samples are taken in 10 min intervals for 1 h• All samples including the original drug solution will be assayed by a validated HPLC or LC-MS/MS method with a minimum of a six point calibration curve and three sets of QC samples• ^{14}C-PEG 4000 concentrations will be determined 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• In-study inspection and post-study audit of data and report by QA

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