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**
- 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 ºC, light-sensitive, etc.

**Deliverables**
- Stability of test compound in MES buffer and intestinal perfusate.
- Effective jejunal permeability (Peff) at three concentrations of test compound in the rat in situ perfusion model relative to the high permeability standard metoprolol
- Preliminary BCS permeability classification (High permeability: Peff_{test}/Peff_{metoprolol} > 1)
- Preliminary assessment of concentration dependent permeability and potential for efflux

**Substrate**
- 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**
- 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**
- Perfusion solution containing the drug in 10 mM MES buffer, pH 6.5, 135 mM NaCl, 5 mM KCl, 0.01% 14C-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**
- Effective permeability (Peff) is calculated following published methods

**Quality Control**
- QC review of raw and processed data

---