*InVitro*CYP™ H-class™ Human Liver Microsomes

Product No.	Description	Size
X008061	*InVitro*CYP H-class 10-Donor Pooled Human Liver Microsomes	10 mg
X008062	*InVitro*CYP H-class 10-Donor Pooled Human Liver Microsomes	20 mg
X008063	*InVitro*CYP H-class 10-Donor Pooled Human Liver Microsomes	100 mg
X008064	*InVitro*CYP H-class 25-Donor Pooled Human Liver Microsomes	10 mg
X008065	*InVitro*CYP H-class 25-Donor Pooled Human Liver Microsomes	20 mg
X008066	*InVitro*CYP H-class 25-Donor Pooled Human Liver Microsomes	100 mg
FX008061	*InVitro*CYP H-class 10-Donor Pooled Female Human Liver Microsomes	10 mg
FX008062	*InVitro*CYP H-class 10-Donor Pooled Female Human Liver Microsomes	20 mg
MX008061	*InVitro*CYP H-class 10-Donor Pooled Male Human Liver Microsomes	10 mg
MX008062	*InVitro*CYP H-class 10-Donor Pooled Male Human Liver Microsomes	20 mg
FX008064	*InVitro*CYP H-class 25-Donor Pooled Female Human Liver Microsomes	10 mg
FX008065	*InVitro*CYP H-class 25-Donor Pooled Female Human Liver Microsomes	20 mg
MX008064	*InVitro*CYP H-class 25-Donor Pooled Male Human Liver Microsomes	10 mg
MX008065	*InVitro*CYP H-class 25-Donor Pooled Male Human Liver Microsomes	20 mg
F008084	*InVitro*CYP H-class Individual Female Human Liver Microsomes	10 mg
F008085	*InVitro*CYP H-class Individual Female Human Liver Microsomes	20 mg
M008084	*InVitro*CYP H-class Individual Male Human Liver Microsomes	10 mg
M008085	*InVitro*CYP H-class Individual Male Human Liver Microsomes	20 mg

Product Description:

Liver microsomes provide a readily available and well-characterized biological model for use in CYP enzyme inhibition studies. *InVitro*CYP H-class microsomes are prepared from tissues that are screened and selected for high activity across the panel of relevant cytochrome P450 enzymes, including those CYPs that are traditionally low in activity. This microsome classification provides researchers with enhanced sensitivity on typically low activity CYPs.

Storage: **≤–70°C**

Incubation Procedure:

Liver microsomes require exogenous cofactors for activity. The cofactors used consist of an NADPH-regenerating system (phase I oxidation) or uridine 5'-diphospho-α-D-glucuronic acid (UDPGA; phase II glucuronidation)[1]. Incubations are usually conducted in 50 to 100 mM Tris buffer, but other buffers may be used.

Drug Inhibition
Refer to the FDA guidance[5] for a thorough description of the parameters involved in determining IC_{50}, Ki reversible inhibition, or mechanistic inhibition.

1. Thaw frozen microsomes by placing the vial under cold running water. Once thawed, keep the vial of microsomes in an ice-water bath until use.
2. Prepare NADPH Regenerating System (NRS; 100 mL total for the following procedure; amount may be altered as appropriate).
   a) Combine 2 g sodium bicarbonate ($NaHCO_3$) per 100 mL deionized water to create 2% $NaHCO_3$.

b) To the 2% $NaHCO_3$ add:
   - 1.7 mg/mL NADP (170 mg for 100 mL),
   - 7.8 mg/mL glucose-6-phosphate (780 mg for 100 mL),
   - 6 units/mL glucose-6-phosphate dehydrogenase (600 units for 100 mL).

For best results, use this solution immediately. The solution can be stored at 4°C for up to 8 hours.

3. Determine the final concentration of test article to be used. Probe substrates are to be selective for single P450 enzyme. Refer to the FDA guidance[5] for preferred and acceptable probe substrates and concentrations. Varying test article and probe substrates concentrations may be used to determine Ki. Prepare a 100X stock of the test article and probe substrates in deionized water. If the test article or probe substrates are insoluble in water, then acetonitrile (ACN) is the preferred organic solvent. Always limit the final concentration of ACN to ≤1%.
4. Total reaction mixtures of 1 mL in 16 × 100 mm glass test tubes work well for test article incubations.
   a) Dilute the microsomes to 10X desired concentration (5 to 20 mg/mL) in buffer such that 100 µL of microsome protein solution will be added to the tubes (0.5 to 2.0 mg/mL final protein concentration). It may be necessary to perform preliminary experiments to optimize protein concentration.
   b) Place the test tubes into an ice bath and add 100 µL of diluted microsomes.
   c) Add 630 µL of buffer.
   d) Add 10 µL of 100X test article stock. Before the addition of probe substrate and NRS, the reaction volume should be exactly 740 µL.
   e) Place the test tubes and the NRS separately into a 37°C shaking water bath for 5 minutes, shaking at 150 rpm. A longer incubation may be performed to determine mechanistic inhibition.
   f) Add 10 µL of 100X probe substrate stock to appropriately labeled tube.
   g) Using a repeater pipette, add 250 µL of NRS to each test tube. Start the reaction timer at the addition of NRS to the first sample.
5. Incubate for the desired time (usually 30 to 60 minutes).

References:

1. Guengerich, F. P. Analysis and characterization of enzymes. In *Principles and Methods of Toxicology* (A.W. Hayes, Ed.). Raven Press, New York, **1989**, pp. 777–813.
2. Spatzenegger, M.; Jaeger, W. Clinical importance of hepatic cytochrome P450 in drug metabolism. *Drug Metab. Rev.* **1995**, *27*, 397–417.
3. Bjornsson, T. D.; Callaghan, J. T.; Einolf, H. J.; Fischer, V.; Gan L.; Grimm, S.; Kao, J.; King, S. P.; Miwa, G.; Ni, L.; Kumar, G.; McLeod, J.; Obach, S. R.; Roberts, S.; Roe, A.; Shah, A.; Snikeris, F.; Sullivan, J. T.; Tweedie, D.; Vega, J. M.; Walsh, J.; Wrighton, S. A. The conduct of in vitro and in vivo drug-drug interaction studies: A PhRMA perspective. *J. Clin. Pharmacol.* **2003** *43*, 443–469.
4. Guidance for Industry: Drug Interaction Studies – Study Design, Data Analysis, and Implications for Dosing and Labeling (Draft, **2006**)

Caution: Treat all products containing human and monkey-derived materials a potentially infectious, as no known test methods can offer assurance that products derived from human or monkey tissues will not transmit infectious agents.

All products are for research use only. Do not use in animals or humans. These products have not been approved for any diagnostic or clinical procedures.