**INVITROCYP™ 150-DONOR HUMAN LIVER MICROSOMES**

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<td>X008070</td>
<td>INVITROCYP M-class 150-Donor Pooled Human Liver Microsomes</td>
<td>10 mg</td>
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**PRODUCT DESCRIPTION:**

Human liver microsomes are subcellular fractions that contain drug-metabolizing enzymes including CYP enzymes, flavin monooxygenases, and UDP glucuronyl transferases. INVITROCYP 150-Donor human liver microsomes are designed to exhibit moderate CYP activity, ideal for clearance and metabolite identification studies. BioIVT’s extensive characterization and tissue profiling process guarantees that each lot of INVITROCYP 150-Donor human liver microsomes will provide consistent and reproducible results for your metabolism and clearance studies.

**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). Incubations are usually conducted in 50 to 100 mM Tris buffer, but other buffers may be used.

**DRUG METABOLISM**

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 (NaHCO₃) per 100 mL deionized water to create 2% NaHCO₃.
   b) To the 2% NaHCO₃ add:
      i) 1.7 mg/mL NADP (170 mg for 100 mL),
      ii) 7.8 mg/mL glucose-6-phosphate (780 mg for 100 mL),
      iii) 6 units/mL glucose-6-phosphate dehydrogenase (600 units for 100 mL).

3) For best results, use this solution immediately. The solution can be stored at 4°C for up to 8 hours. If studying phase II conjugation, add to solution 2b:
   a) 1.9 mg/mL UDPGA (190 mg for 100 mL),
b) Note: The pore-forming antibiotic alamethicin may be used to permeabilize the microsomal membranes and activate glucuronidation, allowing free transfer of UDPGA and glucuronide product across the membrane.

4) Determine the final concentration of test article to be used. Prepare a 100X stock of the test article in deionized water. If the test article is insoluble in water, then acetonitrile (ACN) is the preferred organic solvent. Always limit the final concentration of ACN to ≤1%.

5) 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 640 µL of buffer.
   d) Add 10 µL of 100X test article stock. Before the addition of NRS, the reaction volume should be exactly 750 µL.
   e) Place the test tubes and the NRS separately into a 37°C shaking water bath for 5 minutes, shaking at 150 rpm.
   f) 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.

6) Incubate for the desired time (usually 30 to 60 minutes).

REFERENCES:


CAUTION: Treat all products containing human and monkey-derived materials as 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.