HUMAN KIDNEY, INTESTINE AND LUNG MICROSOMES

Product No.	Description*	Size
X02801	Intestine microsomes, pooled human	5 mg
X03801	Kidney microsomes, pooled human	10 mg
X04801	Lung microsomes, pooled human (smoker)	10 mg
X040032	Lung microsomes, pooled human (non-smoker)	10 mg

**Pooled human intestine and kidney microsomes are available as mixed-gender pools. Other configurations are available through our Custom-Cell™ products.*

PRODUCT DESCRIPTION:

Microsomes are subcellular fractions that contain drug-metabolizing enzymes including the cytochrome P450 enzymes, flavin monooxygenases, and UDP glucuronyl transferases. These microsomes have been prepared from human intestine[1], kidney[2-3] and lung[4-5]. Microsomes are a major tool for studying xenobiotic metabolism[6]. Pooled lots of microsomes have been prepared from several donor tissues, enabling use of these products to evaluate "average human" metabolism of a chosen compound.

STORAGE: ≤–70°C

INCUBATION PROCEDURE:

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)[6]. Incubations are usually conducted in 50 to 100 mM Tris buffer. Other buffers may be used, depending on the analytical method requirements.

1) 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:
      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)
   c) For best results, use this solution immediately. The solution can be stored at 4°C for up to 8 hours.
2) If studying phase II conjugation, add to solution 1b:
      i) 1.9 mg/mL UDPGA (190 mg for 100 mL)

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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[7].*

3) For best results, use this solution immediately. The solution can be stored at 4 °C for up to 8 hours. 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%.
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 the 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.
5) Incubate for the desired time (usually 30 to 60 minutes).

REFERENCES:

1. Zhang, Q. Y.; Dunbar, D.; Ostrowska, A.; Zeisloft, S.; Yang, J.; Kaminsky, L. S. Characterization of human small intestinal cytochromes P-450. *Drug Metab. Disp.* **1999**, *27*, 804–809.
2. Soars, M. G.; Riley, R. J.; Findlay, K. A. B.; Coffey, M. J.; Burchell, B. Evidence for significant differences in microsomal drug glucuronidation by canine and human liver and kidney. *Drug Metab. Disp.* **2001**, *29*, 121–126.
3. Amet, Y.; Berthou, F.; Fournier, G.; Dreamo, Y.; Bardou, L.; Cledes, J.; Menez, J. F. Cytochrome P450 4A and 2E1 expression in human kidney microsomes. *Biochem. Pharmacol.* **1997**, *53*, 765–771.
4. Shimada, T.; Yun, C. H.; Yamazaki, H.; Gautier, J. C.; Beaune, P. H.; Guengerich, F. P. Characterization of human lung microsomal cytochrome P-450 1A1 and its role in the oxidation of chemical carcinogens. *Mol. Pharmacol.* **1992**, *41*, 856–864.
5. Wheeler, C. W.; Guenthner, T. M. Spectroscopic quantitation of cytochrome P-450 in human lung microsomes. *J. Biochem. Toxicol.* **1990**, *5*, 269–272.
6. 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.
7. Fisher, M. B.; Campanale, K.; Ackermann, B. L.; VandenBranden, M.; Wrighton, S. A. In vitro glucuronidation using human liver microsomes and the pore-forming peptide alamethicin. **2000**, *28*, 560–566.

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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.

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