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Animal Liver Microsomes

Induced Sprague-Dawley Rat Liver Microsomes

Product No.	Description	Size
M10001	Aroclor 1254-induced	10 mg
M20001	ß-Naphthoflavone-induced	10 mg
M30001	Clofibrate-induced	10 mg
M40001	Dexamethasone-induced	10 mg
M50001	Isoniazid-induced	10 mg
M60001	3-Methylcholanthrene-induced	10 mg
M70001	Phenobarbital-induced	10 mg

Control Animal Liver Microsomes

Product No.	Description	Size
M00001	Male Sprague-Dawley rat	10 mg
F00001	Female Sprague-Dawley rat	10 mg
M00011	Male Fischer 344 Rat	10 mg
F00011	Female Fischer 344 Rat	10 mg
M00021	Male Wistar rat	10 mg
F00021	Female Wistar rat	10 mg

Control Animal Liver Microsomes, continued

Product No.	Description	Size
M00101	Male Dunkin-Hartley guinea pig	10 mg
F00101	Female Dunkin-Hartley guinea pig	10 mg
M00201	Male beagle dog	10 mg
F00201	Female beagle dog	10 mg
M00301	Male cynomolgus monkey	10 mg
F00301	Female cynomolgus monkey	10 mg
M00311	Male rhesus monkey	10 mg
F00311	Female rhesus monkey	10 mg
M00401	Male New Zealand white rabbit	10 mg
F00401	Female New Zealand white rabbit	10 mg
M00501	Male ICR/CD-1 mouse	10 mg
F00501	Female ICR/CD-1 mouse	10 mg
M00511	Male C57BL/6 mouse	10 mg
F00511	Female C57BL/6 mouse	10 mg
M00601	Male Yucatan minipig	10 mg
F00601	Female Yucatan minipig	10 mg
M00611	Male Gottingen minipig	10 mg
F00611	Female Gottingen minipig	10 mg

Product Description:

Liver microsomes are subcellular fractions that contain drug-metabolizing enzymes including the cytochrome P450 enzymes, flavin monooxygenases, and UDP glucuronyl transferases[1]. Liver microsomes are a major tool for studying xenobiotic metabolism, drug-drug interactions, and covalent binding[2–3]. Pooled lots of human microsomes have been prepared from several livers, enabling use of this product to evaluate “average human” metabolism of a chosen compound.

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. 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% $NaHCO_3$.

1450 South Rolling Road, Baltimore, MD 21227 USA **T** +1 410 455 1242 **F** +1 410 455 1245 customerservice@bioreclamationivt.com
Steinweg 3-5, 60313 Frankfurt am Main, Germany **T** +32 2 808 8278 **F** +32 2 706 5017 customerservice@bioreclamationivt.com

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.

2. If studying phase II conjugation, add to solution 1b:
   - 1.9 mg/mL UDPGA (190 mg for 100 mL),

   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].
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. 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.
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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. 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.

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.