

## Human and Animal Liver S9

Human Liver S9		
Product No.	Description	Size
M00962	Individual male human	30 mg
F00962	Individual female human	30 mg
MX008023	Pooled male human	30 mg
FX008023	Pooled female human	30 mg
X008023	Pooled human, mixed sexes	30 mg

Induced Sprague-Dawley Rat Liver S9		
Product No.	Description	Size
M10002	Aroclor 1254-induced	30 mg
M20002	$\beta$ -Naphthoflavone-induced	30 mg
M30002	Clofibrate-induced	30 mg
M40002	Dexamethasone-induced	30 mg
M50002	Isoniazid-induced	30 mg
M60002	3-Methylcholanthrene-induced	30 mg
M70002	Phenobarbital-induced	30 mg

Control Animal Liver S9		
Product No.	Description	Size
M00002	Male Sprague-Dawley rat	30 mg
F00002	Female Sprague-Dawley rat	30 mg
M00012	Male Fischer 344 rat	30 mg
F00012	Female Fischer 344 rat	30 mg
M00022	Male Wistar rat	30 mg
F00022	Female Wistar rat	30 mg
M00102	Male Dunkin-Hartley guinea pig	30 mg
F00102	Female Dunkin-Hartley guinea pig	30 mg
M00202	Male beagle dog	30 mg
F00202	Female beagle dog	30 mg
M00302	Male cynomolgus monkey	30 mg
F00302	Female cynomolgus monkey	30 mg
M00312	Male rhesus monkey	30 mg
F00312	Female rhesus monkey	30 mg
M00402	Male New Zealand white rabbit	30 mg
F00402	Female New Zealand white rabbit	30 mg
M00502	Male ICR/CD-1 mouse	30 mg
F00502	Female ICR/CD-1 mouse	30 mg
M00512	Male C57BL/6 mouse	30 mg
F00512	Female C57BL/6 mouse	30 mg
M00602	Male Yucatan minipig	30 mg
F00602	Female Yucatan minipig	30 mg
M00612	Male Gottingen minipig	30 mg
F00612	Female Gottingen minipig	30 mg

### Product Description:

Liver S9 fractions are subcellular fractions that contain drug-metabolizing enzymes including the cytochromes P450, flavin monooxygenases, and UDP glucuronyl transferases<sup>1</sup>. Liver S9 fractions are a major tool for studying xenobiotic metabolism<sup>2-3</sup>. Pooled lots of human S9 have been prepared from several livers, enabling use of this product to evaluate “average human” metabolism of a chosen compound.

**Storage:**  $\leq -70^{\circ}\text{C}$

### Incubation Procedure:

Liver S9 requires exogenous cofactors for activity. The cofactors used consist of an NADPH-regenerating system (phase I oxidation), uridine 5'-diphospho- $\alpha$ -D-glucuronic acid (UDPGA; phase II glucuronidation), and 3'-phosphoadenosine-5'-phosphosulphate (PAPS; phase II sulfation)<sup>1</sup>. 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 ( $\text{NaHCO}_3$ ) per 100 mL deionized water to create 2%  $\text{NaHCO}_3$ .
  - b) To the 2%  $\text{NaHCO}_3$  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  $4^{\circ}\text{C}$  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),
  - 100 µg/mL PAPS (10 mg for 100 mL). PAPS is extremely labile and should be added immediately prior to incubation.
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 S9 to the desired concentration (5 to 20 mg/mL) in buffer such that 100 µL of S9 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 S9.
  - 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.
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.

**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.