

## M3000.S9 Lot No. 2210124

BALB/c Mouse Liver S9 Fraction

Untreated, Male, Pool of 400

1.0 mL at 20 mg protein / mL

Suspension medium: 50 mM Tris-HCl, 150 mM KCl, 2 mM EDTA

Specific Content and Enzyme Activities		Content / Rate
Cytochrome P450 content	(nmol/mg protein)	0.302
Cytochrome b <sub>5</sub> content	(nmol/mg protein)	0.204
7-Ethoxycoumarin O-dealkylation	(pmol/mg protein/min)	766 ± 59
Glucuronidation of 4-methylumbelliferone	(nmol/mg protein/min)	101 ± 7
CDNB <sup>a</sup>	(nmol/mg protein/min)	1500 ± 20

<sup>a</sup> 1-Chloro-2,4-dinitrobenzene-glutathione conjugation by glutathione S-transferase.

Values for enzyme activities were determined at a single substrate concentration and are mean ± standard deviation of three or more determinations.

To measure cytochrome P450 (CYP) activity, liver S9 samples (0.2 mg/mL) were incubated in triplicate at 37 ± 2°C for 10 minutes in potassium phosphate buffer (50 mM, pH 7.4), containing MgCl<sub>2</sub> (3.0 mM), EDTA (1.0 mM), NADP (1.0 mM), glucose-6-phosphate (5.0 mM), glucose-6-phosphate dehydrogenase (1 Unit/mL) and 7-ethoxycoumarin (500 µM), at the final concentrations indicated. Metabolite formation was determined by validated LC-MS/MS methods with deuterated metabolites as internal standards.

To measure UDP-glucuronosyltransferase (UGT) activity, liver S9 samples (0.1 mg/mL) were incubated in triplicate at 37 ± 2°C for 10 minutes in Tris-HCl (100 mM, pH 7.7 at 37°C), CHAPS (0.5 mM), EDTA (1.0 mM), MgCl<sub>2</sub> (10 mM), D-saccharic acid 1,4-lactone (100 µM), uridine diphosphate-glucuronic acid (8.0 mM) and 4-methylumbelliferone (1 mM), at the final concentrations indicated. Metabolite formation was determined by validated LC-MS/MS methods with deuterated metabolites as internal standards.

To measure glutathione S-transferase activity (GST), liver S9 samples (5 to 50 µg/mL) were incubated in triplicate at 37 ± 2°C for 10 minutes in potassium phosphate buffer (100 mM, pH 6.5), glutathione (1 mM), and CDNB (1 mM), at the final concentrations indicated. Reaction rates are determined by photometric kinetic measurements at 340 nm.

### Animal Information

Species: Mouse  
 Strain: BALB/c  
 Sex: Male  
 Age: ~ 8-11 weeks  
 Vendor: Charles River, Raleigh, NC

Animals were housed in an AAALAC-accredited facility and allowed to acclimate ≥ seven days before use.

Food: Purina 5L79 (*ad libitum*)  
 Water: Automatic watering system (*ad libitum*)  
 Light/dark cycle: 5:00 am - 5:00 pm, light; 5:00 pm - 5:00 am, dark (12-hour light/dark)  
 Temperature: 70°F ± 2°F  
 Humidity: 30-70 %  
 Bedding: Beta Chip (hardwood), NEPCO, Warrensburg, NY  
 Cage: Polycarbonate Shoebox Cage, conventional cage



### Store at -80°C

**CAUTION:** This sample should be considered as a potential biohazard and universal precautions should be followed. Intended for *in vitro* use only.

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**This data sheet serves as a Certificate of Analysis and has been approved by Stephanie Helmstetter, Senior Manager.**

**Signature and Date:** Stephanie Helmstetter 27 May 2022