

## D1000.S9 Lot No. 2310053

Beagle Dog Liver S9 Fraction

Untreated, Male, Pool of 3

1.0 mL at 20 mg protein / mL

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

<i>Specific Content and Enzyme Activities</i>		<i>Content / Rate</i>
Cytochrome P450 content	(nmol/mg protein)	0.243
Cytochrome b <sub>5</sub> content	(nmol/mg protein)	0.084
7-Ethoxycoumarin O-dealkylation	(pmol/mg protein/min)	1060 ± 100
Glucuronidation of 4-methylumbelliferone	(nmol/mg protein/min)	210 ± 22
CDNB <sup>a</sup>	(nmol/mg protein/min)	287 ± 7

<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: Dog; *Canis familiaris*  
 Strain: Beagle  
 Sex: Male  
 Age: >6 months  
 Vendor: Marshall Bioresources, North Rose, NY

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

Food: Nutrena (*ad libitum*)  
 Water: Automatic watering system, tap water (*ad libitum*)  
 Light/dark cycle: Not monitored  
 Temperature: Ranges from 62°-82°F  
 Humidity: Not monitored  
 Cage: Indoor/outdoor run cages, plastic coated rod bottom, sanitized at least every 2 weeks



## 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, Assistant Director.**  
 Signature and Date: Stephanie Helmstetter 22 March 2023