XENOTECH
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Data Sheet
Certificate of Analysis

R1500.S9 Lot No. 2310060

Sprague Dawley (SD) Rat Liver S9 Fraction
Untreated, Female, Pool of 65
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)	0.201
Cytochrome b_5 content	(nmol/mg protein)	0.125
7-Ethoxycoumarin *O*-dealkylation	(pmol/mg protein/min)	197 ± 15
Glucuronidation of 4-methylumbelliferone	(nmol/mg protein/min)	81.0 ± 7.2
CDNB [a]	(nmol/mg protein/min)	599 ± 31

[a] 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 $MgCl_2$ (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), $MgCl_2$ (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:	Rat
Strain:	International Genetic Standard (IGS), Sprague Dawley
Sex:	Female
Age:	~ 8 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.

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