XENOTECH
A BioIVT Company

Data Sheet
Certificate of Analysis

M1000.IS9 Lot No. 2310192

CD1 Mouse Intestine S9 Fraction
Untreated, Male, Pool of 200
1.0 mL at 4 mg protein / mL
Suspension medium: 50 mM Tris·HCl, 150 mM KCl, 1 mM EDTA, 20% glycerol, heparin, PMSF, leupeptin, DTT, aprotinin

Enzyme Activities		***Rate***
NADPH-cytochrome *c* reductase	(nmol/mg protein/min)	55.2 ± 0.8
Midazolam 1'-hydroxylation	(pmol/mg protein/min)	267 ± 21
Testosterone 6β-hydroxylation	(pmol/mg protein/min)	875 ± 49
Glucuronidation of 4-Methylumbelliferone	(nmol/mg protein/min)	74.9 ± 4.4

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

Aprotinin, Leupeptin, and Phenylmethylsulfonyl-fluoride were used in the preparation of this S9 fraction. Subcellular fractions were prepared from duodenal and jejunal tissue.

To measure cytochrome P450 (CYP) activity, intestine S9 (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 testosterone (250 µM) or midazolam (30 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, intestine S9 (0.2 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 (10.0 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.

Animal Information

Species:	Mouse
Strain:	CD1
Sex:	Male
Age:	~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.

This data sheet serves as a Certificate of Analysis and has been approved by Stephanie Helmstetter, Assistant Director.
Signature and Date: Stephanie Helmstetter 24 July 2023