

M1000.IS9 Lot No. 2310134

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)	52.9 ± 0.9
Midazolam 1'-hydroxylation	(pmol/mg protein/min)	195 ± 8
Testosterone 6β-hydroxylation	(pmol/mg protein/min)	530 ± 26
Glucuronidation of 4-Methylumbelliferone	(nmol/mg protein/min)	61.7 ± 6.6

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 MgCl₂ (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), MgCl₂ (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.

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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 May 2023