

M1000.I Lot No. 2210151

CD1 Mouse Intestine Microsomes Untreated, Male, Pool of 200 150 µL at 10 mg protein / mL

Suspension medium: 250 mM sucrose

Enzyme Activities		Rate
NADPH-cytochrome <i>c</i> reductase Testosterone 6β-hydroxylation Midazolam 1'-hydroxylation Glucuronidation of 4-Methylumbelliferone	(nmol/mg protein/min) (pmol/mg protein/min) (pmol/mg protein/min) (nmol/mg protein/min)	92.2 ± 1.8 1410 ± 100 397 ± 36 138 ± 18

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 these microsomes. Subcellular fractions were prepared from duodenal and jejunal tissue.

To measure cytochrome P450 (CYP) activity, intestine microsomes (0.1 mg/mL) were incubated in triplicate at $37 \pm 2^{\circ}$ 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 μ M) or midazolam (30 μ M), 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 microsomes (0.2 mg/mL) were incubated in triplicate at $37 \pm 2^{\circ}$ 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 μ M), 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.

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: $70^{\circ}F \pm 2^{\circ}F$ Humidity: $30-70^{\circ}M$

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: <u>Stephania Helmstetta</u> 17 June 2022