

D1000.IS9 Lot No. 2110215

Beagle Dog Intestine S9 Fraction

Untreated, Male, Pool of 6

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)	12.3 ± 0.4
Testosterone 6β-hydroxylation	(pmol/mg protein/min)	63.0 ± 3.0
Midazolam 1'-hydroxylation	(pmol/mg protein/min)	65.9 ± 1.7
Glucuronidation of 4-Methylumbelliferone	(nmol/mg protein/min)	3.73 ± 0.34

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 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 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), 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 samples (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 (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: Dog; *Canis familiaris*
 Strain: Beagle
 Sex: Male
 Age: >6 months
 Vendor: Covance, Cumberland, VA

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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