

## P2000.S9 Lot No. 1510192

Cynomolgus Monkey Liver S9 Fraction

Untreated, Male, Pool of 6

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)	0.249
Cytochrome b <sub>5</sub> content	(nmol/mg protein)	0.076
7-Ethoxycoumarin O-dealkylation	(nmol/mg protein/min)	658 ± 89
Glucuronidation of 4-methylumbelliferone	(nmol/mg protein/min)	119 ± 9
CDNB <sup>a</sup>	(nmol/mg protein/min)	1780 ± 40

<sup>a</sup> 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 ± 1°C for 10 minutes in potassium phosphate buffer (50 mM, pH 7.4), containing MgCl<sub>2</sub> (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 ± 1°C for 10 minutes in Tris-HCl (100 mM, pH 7.7 at 37°C), CHAPS (0.5 mM), EDTA (1.0 mM), MgCl<sub>2</sub> (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 ± 1°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: Monkey  
 Strain: Cynomolgus  
 Sex: Male  
 Age: 2-3 years  
 Vendor: Charles River, Reno, NV

Animals were housed in an AAALAC-accredited facility and allowed to acclimate ≥ seven days before use.

Imported animals were quarantined for one month prior to shipment into the United States to reduce the risk of importing Ebola virus-infected monkeys. All animals were under veterinary care and were asymptomatic at the time of euthanasia. All of the monkeys tested negative for Simian Retrovirus. None of the animals examined tested positive for any other infectious agents



### 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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