Corning® Gentest™ Human Liver Microsomes Allelic Variant- 3A5*3*3

Product Description	
Catalog No.	452137
Lot No.	HH189
Donor No.	HH189
Qty./Package	0.5 ml
Protein Content	20 mg/ml in 250 mM sucrose
Storage Conditions	Store at -80 C
Release Date	2007 September

Assay Results

Enzyme Measured	Assay[a]	Enzyme Activity [in pmol/(mg x min)]
Total P450[c]	Omura and Sato	300 pmol/mg
OR	Cytochrome c Reductase	250
Cytochrome b_5	Spectrophotometric	420 pmol/mg
CYP1A2	Phenacetin O-deethylase	230
CYP2A6	Coumarin 7-hydroxylase	1200
CYP2B6	(S)-Mephenytoin N-demethylase	48
CYP2C8	Paclitaxel 6α-hydroxylase	210
CYP2C9	Diclofenac 4'-hydroxylase	4100
CYP2C19	(S)-Mephenytoin 4'- hydroxylase	270
CYP2D6[b]	Bufuralol 1'-hydroxylase (The amount of activity inhibited by 1 µM quinidine.)	37
CYP2E1	Chlorzoxazone 6-hydroxylase	2500
CYP3A4	Testosterone 6β-hydroxylase	13600
CYP4A11	Lauric acid 12-hydroxylase	1800
FMO	Methyl p-Tolyl Sulfide Oxidase	2000
UGT1A1	Estradiol 3-Glucuronidation	560
UGT1A4	Trifluoperazine Glucuronidation	700
UGT1A9	Propofol Glucuronidation	2400
Western Blot Results[d]		
CYP3A5	Western blot	n.d.
CYP3A4	Western blot	120 pmol/mg

a. All cytochrome P450 assays conducted at 0.8 mg/ml protein (except CYP3A4 which was at 0.5 mg/ml) with an NADPH generating system (1.3 mM NADP, 3.3 mM glucose 6-phosphate and 0.4 U/ml glucose 6-phosphate dehydrogenase), 3.3 mM $MgCl_2$, and incubated for 20 minutes or 10 minutes (CYP2C8, CYP2C9, CYP3A4 and CYP4A). 0.1 M Potassium phosphate buffer (pH 7.4) was used for all P450 enzymes except CYP2B6 and CYP2C19 (0.05 M) and CYP2A6, CYP2C9 and CYP4A which used 0.1 M Tris (pH 7.5). The FMO assay was conducted in the same volume and protein concentration in 0.05 M glycine buffer (pH 9.5) with the same NADPH generating system, 3.3 mM $MgCl_2$, 1.2 mM diethylenetriaminepentacetic acid, 0.5 mg/ml Triton X-100 and incubated for 10 minutes. All UGT Glucuronidation assays contained 0.5 mg/ml protein (except UGT1A9 which was at 0.15 mg/ml), 2 mM UDPGA, 10 mM $MgCl_2$, 25 ug/ml Alamethicin in 50 mM Tris-HCl buffer (pH 7.5). UGT1A1 was incubated for 30 minutes, 1A4 for 20 minutes, and 1A9 for 10 minutes. Activities expressed as pmol product per (mg protein x minute) except cytochrome c reductase which is expressed as nmole product per (mg protein x minute).

b. The amount of activity inhibited by 1 uM quinidine.

c. pmol P450/mg of total protein

d. The Western Blot assay was carried out using standard protocols. SDS-gel electrophoresis was by the method of Laemmli (Laemmli, U.K, 1970, *Nature*, 227: 680-685.). CYP protein abundance in HLM was quantitated using authentic standards derived from recombinant P450 isoforms.

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For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Donor Information

Specimen	HH189
Gender	Female
Age	54
Race	Caucasian
Cause of Death	CVA
Medical History	MVI and Calcium
Medication given during Hospitalization	Nipride, Insulin, Norepinephrine, Vasopressin, Unasyn, Levaquin, Ancef, Heparin, Levophed

Donor Genotypes

Specimen	Genotype
HH189	3A5*3*3

HLM donors with 1*1 and *1*3 genotypes have high CYP3A5 protein levels whereas *3*3 have low CYP3A5 protein levels. The CYP3A5*3 defective allele is caused by a single nucleotide polymorphism in intron 3 leading to the insertion of an intronic sequence containing premature termination codons, and hence the very low CYP3A5 protein expression in subjects with the *3*3 genotype.

Pathogenicity Testing by Polymerase Chain Reaction (PCR)

Specimen	HH189
HIV1	**–**
HIV2	**–**
HTLV1	**–**
HTLV2	**–**
HBC	**–**
HCV	**–**
CMV	**–**

* Donor was serologically positive for CMV. ****** Serology unknown.

HAZARD WARNING:
This microsome preparation was prepared from freshly frozen human tissues. These materials have tested negative for HIV, HTLV and hepatitis. However, we recommend that this material be considered a potential biohazard.

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