

# BD UltraPool™ HLM 150 Characterization

- ✓ Single substrate concentration activity
- ✓ Scaling factor
- ✓ Western blot
- ✓ Enzyme kinetics

## Lot 38290

Enzyme Measured	Assay	Specific Content or Activity
Total P450	Omura and Sato	350 pmol/mg
OR	Cytochrome c Reductase	270 nmol/min*mg
Cytochrome b <sub>5</sub>	Spectrophotometric	520 pmol/mg

CYP Isoform	Probe Substrate Activity	[S] (μM)	Activity (pmol/min*mg)
CYP1A2	Phenacetin <i>O</i> -dealkylation	100	690
CYP2A6	Coumarin 7-hydroxylation	100	830
CYP2B6	( <i>S</i> )-Mephenytoin <i>N</i> -demethylation	100	40
CYP2C8	Paclitaxel 6α-hydroxylation	100	170
CYP2C9	Diclofenac 4'-hydroxylation	100	3100
CYP2C19	( <i>S</i> )-Mephenytoin 4'-hydroxylation	100	57
CYP2D6	Bufuralol 1'-hydroxylation (Amount of activity inhibited by 1 μM quinidine)	25	71
CYP2E1	Chlorzoxazone 6-hydroxylation	1000	2400
CYP3A4	Testosterone 6β-hydroxylation	200	4600
CYP4A11	Lauric acid 12-hydroxylation	100	1700
FMO	Methyl <i>p</i> -Tolyl Sulfide Oxidation	1000	1100
UGT1A1	Estradiol 3-Glucuronidation	100	940
UGT1A4	Trifluoperazine <i>N</i> -Glucuronidation	100	580
UGT1A6	Serotonin Glucuronidation	1000	10000
UGT1A9	Propofol Glucuronidation	20	3700
UGT2B7	AZT Glucuronidation	1000	760

- Methods: 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<sub>2</sub>, 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, CYP2C8, 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<sub>2</sub>, 1.2 mM diethylenetriaminepentaacetic acid, 0.5 mg/mL Triton™ X-100 and incubated for 10 minutes. UGT Glucuronidation assays contained 0.5 mg/mL protein for UGT1A1 and 1A4, 0.1 mg/mL for 1A6, 0.15 mg/mL for 1A9 and 0.8 mg/mL for 2B7, along with 2 mM UDPGA, 10 mM MgCl<sub>2</sub>, 25 ug/mL Alamethicin in 50 mM Tris-HCl buffer (pH 7.5). UGT1A1 was incubated for 30 minutes, 1A4 for 20 minutes, 1A6 for 15 minutes, 1A9 for 10 minutes and 2B7 for 25 minutes.

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## Microsome Scaling Factor in 150 Donor Pool

mg Microsomal Protein/gr Liver
43

- Methods: Scaling factor was determined by the Matsubara method (Anal. Biochem., vol. 75, p. 596, 1976).

## CYP Isoform Abundance in 150 Donor Pool

Enzyme Measured	Assay	CYP Abundance (pmol / mg)
CYP3A4	Western Blot	67
CYP3A5	Western Blot	21

- Methods: 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 the pooled HLM was quantitated using authentic standards derived from recombinant P450 isoforms and purified proteins.

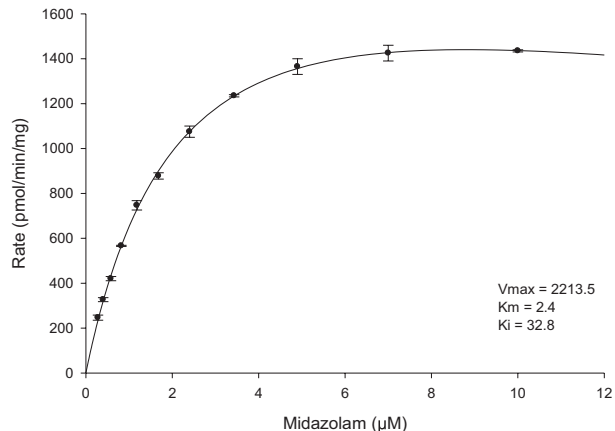
## Kinetic Constants for CYP Isoform Activities for BD UltraPool™ HLM 150

Enzyme Measured	Substrate	Assay Protein Con. (mg/mL)	Assay Time (min)	[S] Range	Km (μM)	Vmax [pmol/(mg x min)]	Regression Model
CYP1A2	Phenacetin	0.2	10	6.8-240 μM	41	912	Michaelis-Menten
CYP2A6	Coumarin	0.05	5	0.28-10 μM	1.1	529	Michaelis-Menten
CYP2B6	Bupropion	0.1	5	13-450 μM	73	377	Michaelis-Menten
CYP2C8	Amodiaquine	0.02	5	0.28-10 μM	1.5	2215	Michaelis-Menten
CYP2C9	Diclofenac	0.05	5	0.85-30 μM	5.3	2313	Michaelis-Menten
CYP2C19	(S)-Mephenytoin	0.3	10	6.8-240 μM	15	74	Michaelis-Menten
CYP2D6	Dextromethorphan	0.1	5	0.42-15 μM	1.5	180	Michaelis-Menten
CYP2E1	Chlorzoxazone	0.1	5	10-360 μM	55	3143	Michaelis-Menten
CYP3A4	Midazolam	0.02	5	0.28-10 μM	2.4	2214	Substrate Inhibition
CYP3A4	Testosterone	0.05	10	8.5-300 μM	75	6796	Hill (n=1.1)

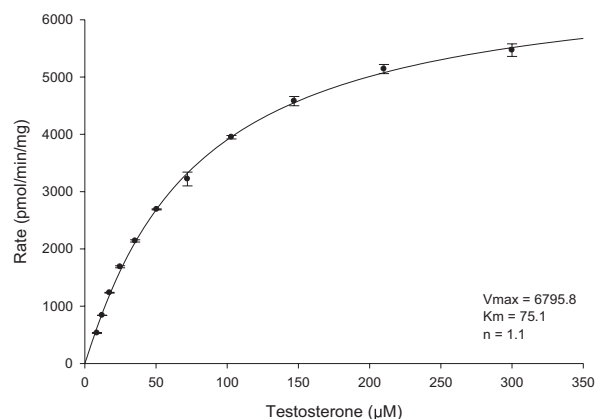
- Methods: Incubations for Km and Vmax determination contained 100 mM Phosphate (pH 7.4), 3.3 mM MgCl<sub>2</sub>, NADPH generating system (1.3 mM NADP, 3.3 mM glucose 6-phosphate and 0.4 U/mL glucose 6-phosphate dehydrogenase) and CYP probe substrate (10 to 12 concentrations evenly spaced over the range). Metabolite formation was analyzed using validated LC-MS/MS methods with stable isotope labeled metabolites as internal standards.

# Kinetic Analysis Graphs: $K_m$ ( $\mu\text{M}$ ) and $V_{\text{max}}$ ( $\text{pmol}/\text{min}\cdot\text{mg}$ ) for BD UltraPool™ HLM 150

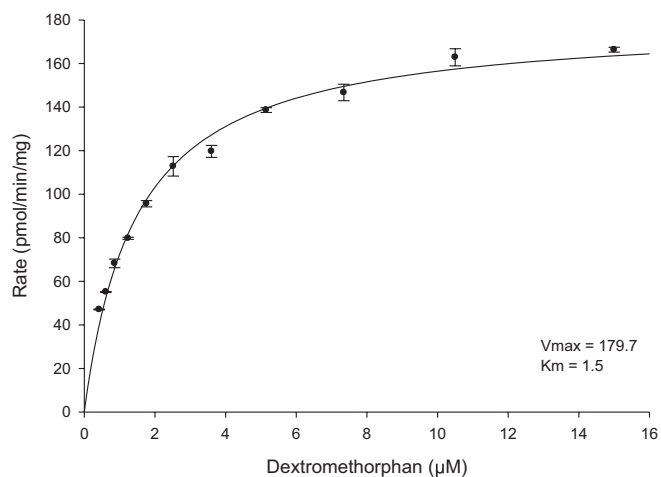
## CYP3A4-Midazolam 1'-hydroxylation



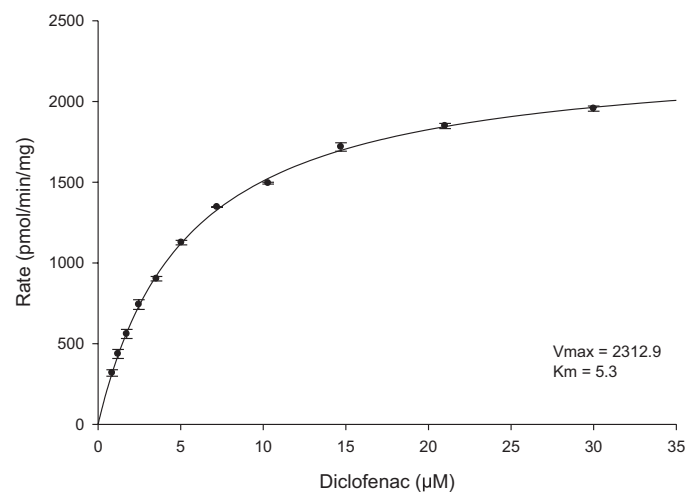
## CYP3A4-Testosterone 6 $\beta$ -hydroxylation



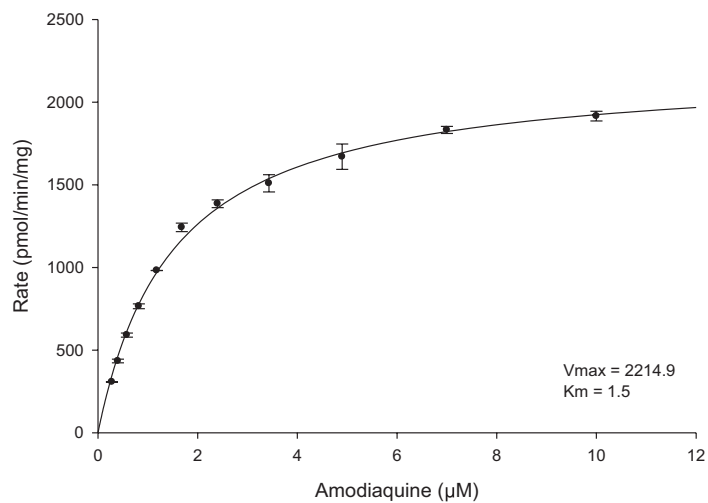
## CYP2D6-Dextromethorphan O-demethylation



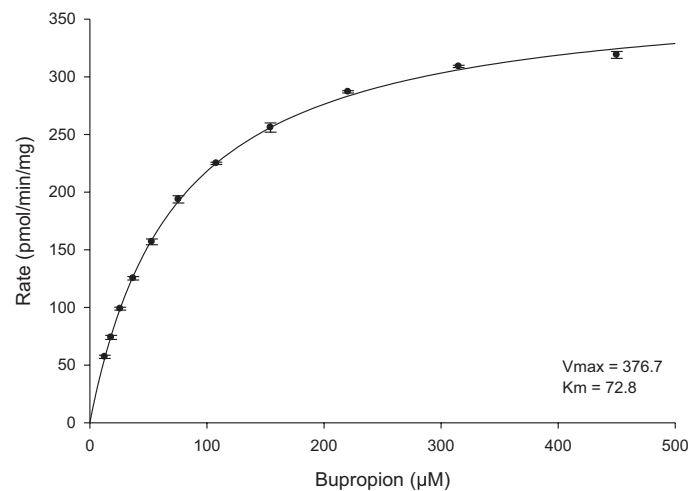
## CYP2C9-Diclofenac 4'-hydroxylation



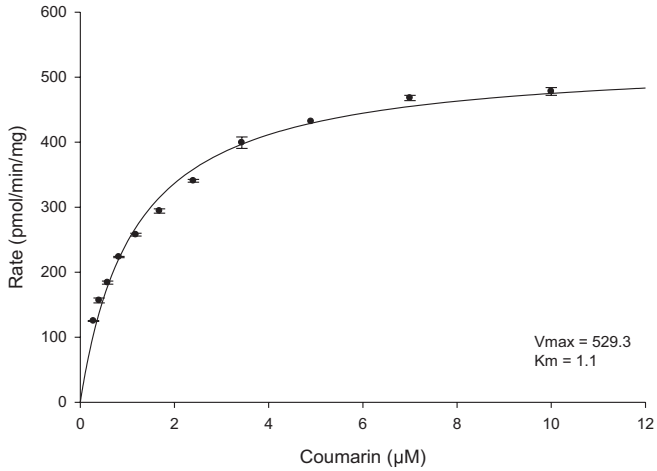
## CYP2C8-Amodiaquine N-dealkylation



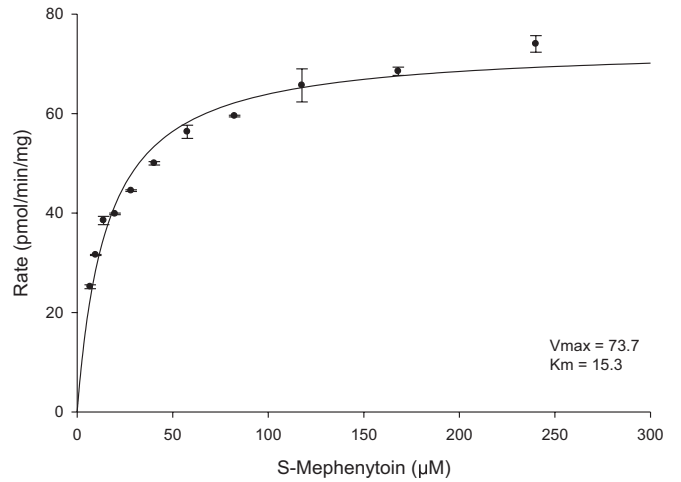
## CYP2B6-Bupropion hydroxylation



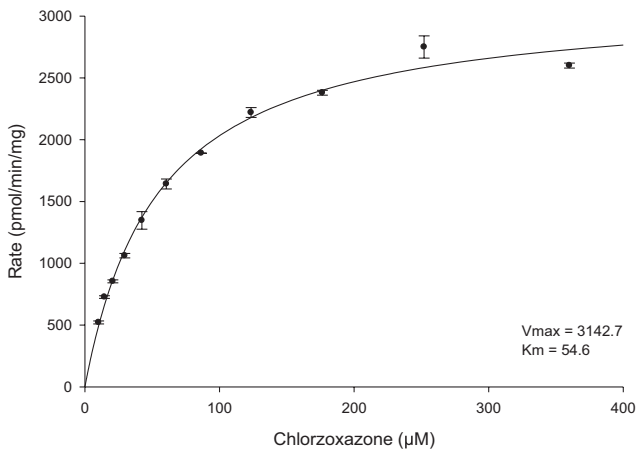
### CYP2A6-Coumarin hydroxylation



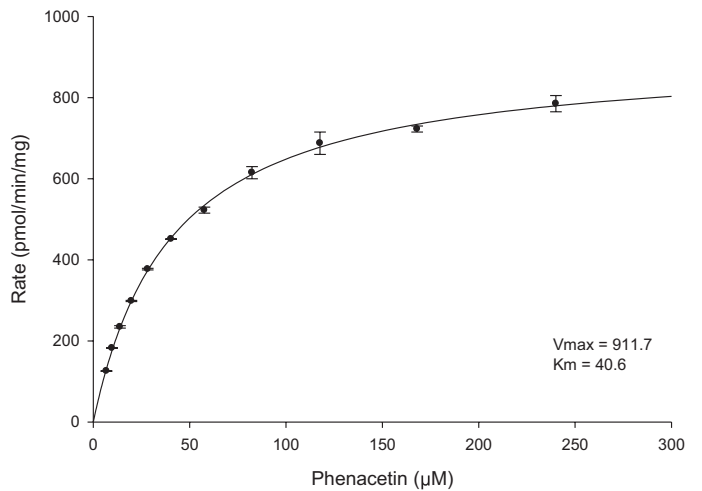
### CYP2C19-S-Mephenytoin 4'-hydroxylation



### CYP2E1-Chlorzoxazone 6-hydroxylation



### CYP1A2-Phenacetin O-dealkylation



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