

INVENTORY DATASHEET

BD Gentest™ Plateable Cryopreserved Human Hepatocytes

Donor availability as of April 9, 2012. Availability after this date may differ from this chart. Call your local BD Representative for the most up-to-date information.

BD Lot No.	Application(s)	Gender	Age (years)	Race	Smoker	Viability (%)	Recovery (10 ⁶ cells/vial)	Transporter activity (pmol/min/mg)		Intrinsic clearance (mL/hr/10 ⁶ cells)		Metabolite formation velocity (nmol/hr/10 ⁶ cells)			Basal activity (pmol/min/mg)	Induced activity (pmol/min/mg)	Fold induction	Basal activity (pmol/min/mg)	Induced activity (pmol/min/mg)	Fold induction
								Taurocholate Uptake	Rosuvastatin Uptake	CYP3A4 (Midazolam)	CYP2D6 (Dextromethorphan)	CYP1A2 (Phenacetin)	CYP2C9 (Diclofenac)	UGT1A1 (Estradiol)	CYP3A4	CYP3A4	CYP3A4	CYP1A2	CYP1A2	CYP1A2
Transporter-Qualified Cat. No. 454541 ≥ 5 Million cells/vial																				
195	T (I)	F	2	H	N	90	9.4	13.4	5.1					10.0	1410.0	140	11	81	7	
276	T (I)	F	59	AA	Y	90	8.0	19.7	11.3					19.0	1200.0	64	ND	ND	ND	
295	T (I)	F	41	C	Y	90	6.4	13.2	5.8					42	1800	43	30	460	15	
312	T (I)	M	56	C	Y	85	5.7	12	7.7					12.0	760.0	63	8.2	240	29	
317	T (I)	M	65	C	N	81	8.0	12	3.5					43.0	1100.0	26	2	120	60	
318	T (I)	M	58	AA	N	84	8.0	6.2	1.8					31.0	1300.0	42	1.7	160	94	
Metabolism-Qualified Cat. No. 454543 ≥ 5 Million cells/vial																				
261	M (I)	M	20	C	Y	77	5.0			0.302	0.258	2.3	3.6	0.78	140.0	1600.0	11	22	160	7
281	M (I)	F	49	C	Y	90	7.7			0.221	0.124	1.37	2.38	1.11	59.0	1330.0	23	6.2	130	21
303	M (I)	F	52	C	N	89	5.4			0.25	0.457	0.26	2.8	0.83	36.0	1600.0	44	6.8	210	31
304	M (I)	M	64	C	N	90	8.4			0.262	0.69	0.4	10.1	0.95	55.0	860.0	16	4.3	180	42
330	M (I)	F	49	C	Y	85	6.4			0.33	2.6	0.76	32	0.46	120.0	1500.0	13	4	110	48
Inducible-Qualified Cat. No. 454550 2-5 Million cells/vial																				
118	I	M	42	C	Y	87	4.9								ND	ND	13	ND	ND	2
142	I	M	48	C	N	95	6.5								50	411	10	2	23	13
170	I	F	63	C	Y	86	3.5								73.0	354.0	5	6	10	2
258	I	M	53	C	N	86	3.9								27.0	110.0	4	ND	ND	ND
264	I	F	45	C	Y	91	7.2								4.0	410.0	100	2	19	10
277	I	F	41	C	Y	89	7.0								6.6	390.0	57	6	50	21
290	I	F	69	C	Y	84	4.8								10.0	450.0	45	2	36	18
299	M (T, I)	F	20	H	Y	90	3.8	10.4	9.1	0.58	0.53	0.22	2.5	1.07	290.0	1800.0	6	32	300	9
307	I	M	29	C	Y	91	4.4								9.7	930.0	96	4	93	24
309	I	M	69	C	Y	88	4.4								18.0	1200.0	67	11	200	18
Inducible-Qualified Cat. No. 454551 ≥ 5 Million cells/vial																				
107	I	F	55	C	N	81	6.2								ND	ND	8	ND	ND	9
141	I	M	3 months	C	N	88	14.9								ND	ND	5.4	ND	ND	4.8
166	I	F	71	C	Y	93	7.9								36.4	273.0	7.5	0.47	18.5	39
178	I	F	58	C	Y	90	8.9								5.2	335.0	65	0.65	24	37
178*	I	F	58	C	Y	90	7.6								4.4	680.0	160	4.7	86	18
226	I	F	5 months	C	N	80	17.3								15.0	580.0	40	5.2	43	8.3
228	I	F	43	C	Y	90	7.5								81.0	1200.0	15	11	183	17
246	I	M	4	C	N	86	12.9								42.0	1160.0	27.6	26	1180	45.4
260	I	M	5	AA	N	90	10.1								28.0	530.0	19	ND	ND	ND
266	I	M	64	C	Y	88	6.9								12.0	92.0	7.7	ND	ND	ND
267	I	F	63	C	N	83	6.5								16.0	750.0	47	1	34	34
271	I	F	64	C	N	88	7.9								36.0	800.0	22	ND	ND	ND
276	I	F	59	AA	Y	90	8.0								19.0	1200.0	64	ND	ND	ND
279	I	M	28	C	Y	81	7.3								9.0	440.0	50	ND	ND	ND
281	I	F	49	C	Y	90	7.7								59.0	1300.0	23	6.2	130	21
285	I	M	61	C	Y	95	7.7								16.0	1100.0	69	2.1	96	46
288	I	M	58	C	Y	79	7.8								14.0	510.0	36	2.9	27	9.3
302	I	M	33	C	Y	81	5.4								16.0	680.0	43	3.9	64	16
303	I	F	52	C	N	89	5.4								36.0	1600.0	44	6.8	210	31
304	I	M	64	C	N	90	8.4								55.0	860.0	16	4.3	180	42
311	I	M	75	C	N	81	8.6								16.0	1200.0	75	4.8	160	33
314	I	F	35	C	N	93	5.3								31.0	1600.0	52	4.7	71	15
314A	I	F	35	C	N	93	6.3								21.0	1200.0	57	4.3	53	12
318	I	M	58	AA	N	84	8.0								31.0	1300.0	42	1.7	160	94
319	I	M	53	C	N	87	7.2								11.0	360.0	33	2.1	120	57
320	I	F	61	C	Y	80	7.5								11.0	220.0	20	2.4	57	24
321	I	F	58	C	Y	85	7.3								21.0	1800.0	86	8.9	250	28
325	I	M	66	C	Y	82	6.6								34.0	590.0	17	3.6	90	25
94	I	M	13	H	N	83	5.1								ND	ND	9	ND	ND	18

Characterization Key

RACE: A: Asian ; AA: African American ; C: Caucasian ; H: Hispanic ; NA: Native American

APPLICATION: I: Inducible ; M: Metabolism ; T: Transporter

The first application listed is the primary application for this lot of hepatocytes, and the applications in parenthesis () are the secondary applications.

ND: Not determined

U.S. Orders: tel:

Purchase Orders should be made out to: BD Biosciences, 2350 Qume Drive, San Jose, CA 95131

Technical Support: tel: 877.232.8995 ; e-mail: admtox@bd.com



INVENTORY DATASHEET

Assay Conditions

	Assay	Enzyme or transporter	Positive control inducer	Substrate	Metabolism reaction measured
TRANSPORTER	Efflux Transport	MRP2	NA	5 μ M CDFDA (hydrolysis product CDF, 5-(and-6)-carboxy-2',7'-dichlorofluorescein)	NA
	Uptake Transport	NTCP	NA	1 μ M Taurocholic Acid	NA
		OATP1B1/ OATP1B3	NA	2 μ M Rosuvastatin	NA
METABOLISM	Intrinsic Clearance	CYP3A4	NA	0.5 μ M Midazolam	Midazolam 1-hydroxylation
		CYP2D6	NA	1 μ M Dextromethorphan	Dextromethorphan O-Demethylation
	Metabolite Formation	CYP1A2	NA	100 μ M Phenacetin	Phenacetin O-deethylation
		CYP2C9	NA	100 μ M Diclofenac	Diclofenac 4-hydroxylation
		UGT1A1	NA	100 μ M Estradiol	Estradiol 3-glucuronidation
INDUCTION	Induction	CYP1A2	20 μ M β -Naphthoflavone	100 μ M Phenacetin	Phenacetin O-deethylation
		CYP3A4	20 μ M Rifampicin	200 μ M Testosterone	Testosterone 6 β -hydroxylation

Cell Culture Procedure

Thawing and Plating CryoHepatocytes

The viability and recovery for these lots of cryopreserved hepatocytes were evaluated using the BD Gentest™ High Viability CryoHepatocyte Recovery Kit (Cat. No. 454534). This medium is a proprietary non-cytotoxic recovery medium which requires a single centrifugation step.

Individual Assay Procedure

Plated Transporter Assay

After thawing, cells were plated at a concentration of 0.4 x 10⁶ cells/well on a BD BioCoat Collagen I 24-well plate (Cat. No. 356408). Two to four hours after cell plating the media was changed to BD Gentest™ CryoHepatocyte Plating Medium (Cat. No. 454561) with 10% Fetal Bovine Serum (FBS), and cells were incubated overnight at 37°C with 5% CO₂. After 18 - 24 hours post plating, cells were overlaid with 0.25 mg/mL BD Matrigel™ Matrix (Cat. No. 356237) prepared in supplemented William E Medium. On day 3 and day 4, media changes were conducted with fresh supplemented William E Medium. On day 5, bile canaliculi formation and uptake assays were conducted with the appropriate substrate.

Plated Metabolism Assay

After thawing, cells were plated at a concentration of 0.168 x 10⁶ cells/well on a BD BioCoat™ Collagen I 48-well plate (Cat. No. 356505). Two to four hours after cell plating, media was changed to supplemented William E Medium and cells were incubated overnight at 37°C with 5% CO₂. After 18 - 24 hours post plating, cells were incubated with substrate/test compound for 8 hours or overnight. During incubation, samples were taken at different time points (e.g., 1, 2, 3, 4, 6, 8 hours) to determine parent compound loss (Intrinsic Clearance) or metabolite formation (Velocity) by LC-MS/MS analysis.

Induction Assay

After thawing, cells were plated at a concentration of 0.4 x 10⁶ cells/well on a BD BioCoat™ Collagen I 24-well plate (Cat. No. 356408). Two to four hours after cell plating, media was changed to BD Hepatocyte Culture Media with FBS (Cat. No. 355056) and cells were incubated overnight at 37°C with 5% CO₂. On day 2, cells were induced with either 20 μ M Rifampicin or 20 μ M β -Naphthoflavone every 24 hours over a 3-day period. Control cells were induced with appropriate solvent over the same time period. Enzyme assays were conducted on day 5. Incubations were performed in BD Hepatocyte Culture Media with the appropriate P450 substrate. Testosterone (CYP3A4) and Phenacetin (CYP1A2) assays were terminated after 30 minutes and 60 minutes of incubation, respectively, and analyzed by HPLC.

