

March 7, 2013

Certificate of Analysis
Plateable CryoHeps
Lot # B0403VT

• **Product**

Process	Human Hepatocytes were isolated and frozen according to KaLy-Cell's SOPs
Biosafety level	Refer to the attached MSDS
Freezing date	March 05, 2010

For In Vitro use only, not to be used for clinical application. KaLy-Cell cell products may contain human material that should be treated as potentially hazardous. Use universal precautions for handling biohazardous.

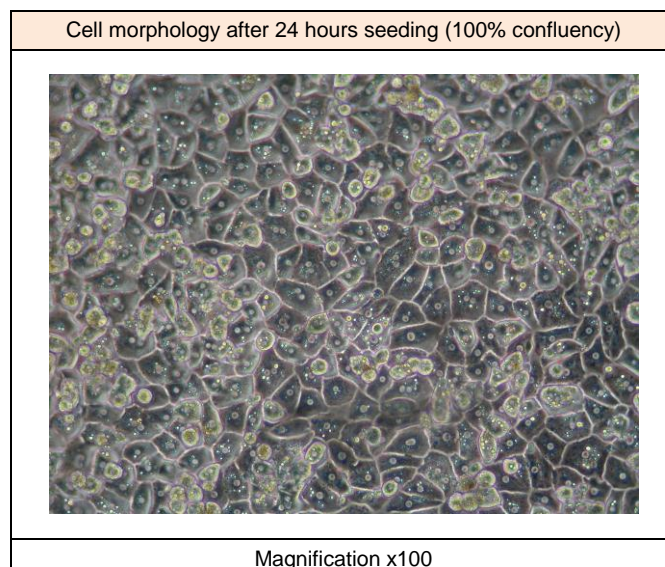
• **Donor information**

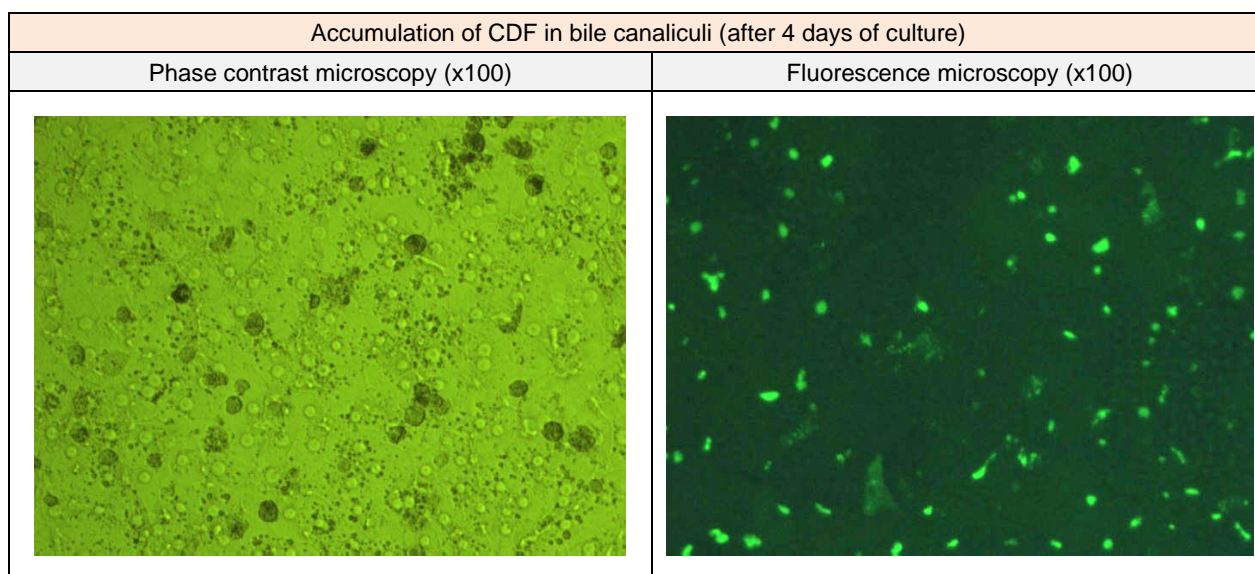
Sex, Age	Female, 47 years old	
Pathology	None	
Medication	Unknown	
Race	Caucasian	
Hepatitis B : Negative	Hepatitis C : Negative	HIV-1/2 : Negative

• **Batch informations**

	Acceptability criteria	Lot Result
Number of viable cells/vial	$\geq 2 \times 10^6$	7.6×10^6
Post-thaw viability	$\geq 80 \%$	88 %
Plateability (after 24hours seeding)	$\geq 80 \%$	100 %
Seeding Density in 48 well plate	0.20-0.25 x 10^6 viable cells per well	0.25×10^6
Seeding Density in 96 well plate	0.07×10^6 viable cells per well	0.07×10^6

*Human Hepatocytes were thawed and seeded according to KaLy-Cell's SOPs. The post-thaw yield and viability (trypan blue exclusion) of Hepatocytes were assessed after a purification process.
 Plateability was assessed by seeding on Rat Tail Collagen Type I home coated plates*





- **Characterization in suspension**

- **CYPs activities** expressed in pmol/min/10⁶cells

Activity	CYP involved	Lot Result	Historical Data					n
			Mini	Low	Medium	High	Max	
Ethoxyresorufin – O –deethylation	CYP1A2	5.16	0	< 1.80	1.80 - 6.30	> 6.30	30.8	173
6β-testosterone hydroxylation	CYP3A4/5	11.3	0.108	< 14	14 - 45	> 45	159	137
Bupropion hydroxylation	CYP2B6	71.2	0.156	< 11.4	11.4 - 38.5	> 38.5	129	173
Tolbutamide hydroxylation	CYP2C9	ND	0.065	< 7	7 - 40	> 40	179	117
Bufuralol hydroxylation	CYP2D6	4.24	0.0537	< 4	4-11	> 11,4	32.2	82

- **Clearance** of the substrates for the phase I enzyme expressed in μL/min/10⁶cells

Substrates	CYP involved	Lot Result	Historical Data					n
			Mini	Low	Medium	High	Max	
Midazolam	CYP3A4/5	6.49	0.135	< 4	4 - 10	> 10	22	88
Diclofenac	CYP2C9	6.90	0.811	< 7	7 - 18	> 18	14.9	42
Bufuralol	CYP2D6	6.20	0.629	< 4.50	4.50 - 14	> 14	43.8	31

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• **Characterization in monolayer**

- **CYPs activities** 24h after seeding expressed in pmol/min/mg protein

Activity	CYP involved	Lot Result	Historical Data					n
			Mini	Low	Medium	High	Max	
Ethoxyresorufin – O –deethylation	CYP1A2	1.78	0.018	< 1	1 - 3	> 3	34.9	77
6β-testosterone hydroxylation	CYP3A4/5	80.1	1.42	< 68	68 - 250	> 250	653	46
Bupropion hydroxylation	CYP2B6	ND	2.92	< 27	27 - 55	> 55	204	31
Diclofenac 4-hydroxylation	CYP2C9	ND	0.925	< 20	20 - 55	>55	88.5	31

- **CYPs activities** (expressed in pmol/min/mg protein) and **fold induction** after 3 days of treatment

Activity	CYP involved	Inducers	Control	Induced	Fold Induction	Acceptability criteria*
Ethoxyresorufin – O –deethylation	CYP1A2	β-naphoflavone 50 µM	1.56	12.9	8	5
Midazolam 1'-hydroxylation	CYP3A4/5	Rifampicin 10 µM	15.4	47.4	3	2
Bupropion hydroxylation	CYP2B6	Phenobarbital 1000 µM	13.7	140.8	10	2
Tolbutamide hydroxylation	CYP2C9	Rifampicin 10 µM	0.700	9.90	14	2

*: response to inducers according to FDA, DDI guidance for industry, 2006 and Richert et al, 2009

• **Cell storage and use**

Delivery	In dryshipper with nitrogen vapor phase
Storage	In liquid nitrogen or -150°C tank
Instruction for using the cells	Follow KaLy-Cell's Thawing protocol provided with the cells


• **Companion products**

Reference	Denomination
KLC-TWM	Kit KLC Thawing & Washing medium
KLC-SM	KLC Seeding Medium
KLC-IM kit	KLC-IM : KLC Maintenance Medium KLC-AD1 : Additive 1 KLC-AD2 : Additive 2
KLC-AD3	Additive 3 Maintenance Medium (for long term culture)
KLC-PLA	Home-coated Plates (all formats)

To place an order or inquire about our products and services please contact by phone 0033 88108831 or by e-mail : l.richert@kaly-cell.com or e.alexandre@kaly-cell.com

ND = Not done

VISA FOR BATCH RELEASE:

Name Alexandre Eliane	Function Laboratory Manager	Qualification Engineer	Signature 	Date March 7, 2013
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- Alexandre, E., Baze, A., Parmentier, C., Desbans, C., Pekthong, D., Gerin, B., Wack, C., Bachelier, P., Heyd, B., Weber, J.-C., Richert, L., 2012. Plateable cryopreserved human hepatocytes for the assessment of cytochrome P450 inducibility: experimental condition-related variables affecting their response to inducers. *Xenobiotica* 42, 968-979.
- Richert L, Tuschl G, Abadie C, Blanchard N, Pekthong D, Manton G, Weber J-C, Mueller S-O, 2009, Use of mRNA expression to detect the induction of drug metabolizing enzymes in rat and human hepatocytes. *Toxicology and Applied Pharmacology* 235, 86-96.
- Guidance for industry, Drug Interaction Studies - Study Design, Data Analysis, and Implications for Dosing and Labelin, 2006.