CERTIFICATE OF ANALYSIS

HUMAN SHORT-TERM HEPATOCYTES IN MONOLAYER

Catalog number: HEP200

Batch number: HEP200482

For in vitro use only

For your safety /// Biohazard information /// These biologicals have to be considered as potentially dangerous, take maximum care in order to protect yourself, your colleagues and your environment.

1 BIOLOGICAL MATERIAL

ORIGIN OF CELLS		
Subject, age	Human adult, 81 years old	
Sex	Male ☐ Female ☒	
Ethnicity	Caucasian ☐ African ☐ Unknown ☒	
Liver pathology *(macroscopic observation)*	Hepatic metastases	
Patient information	Diabetes	Yes ☐ No ☒ Unknown ☐
	Heart disease	Yes ☐ No ☐ Unknown ☒
	High blood pressure	Yes ☐ No ☐ Unknown ☒
	Smoking	Yes ☐ No ☒ Unknown ☐
	Alcoholism	Yes ☐ No ☐ Unknown ☒
	Medication: ®, Unknown ☒	

SAFETY DATA		
Virological status	**Specification**	**Result**
Hepatitis B *(HBs antigen, anti HBc antibody)*	Negative	Positive ☐ Negative ☒ In progress ☐
Hepatitis C *(anti HCV antibody)*		Positive ☐ Negative ☒ In progress ☐
HIV-1 and HIV-*2 (anti HIV-1 and -2 antibodies)*		Positive ☐ Negative ☒ In progress ☐
Biosafety level	All human sourced products should be handled at the Biological Safety Level. 2 (BSL 2) to minimize exposure of potentially infectious products	

Virological status *for HIV1-2, HCV, HBV is carried out on the patient by serological screening using approved diagnostic kits.*

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S.A.R.L. au capital de 98 500 euros
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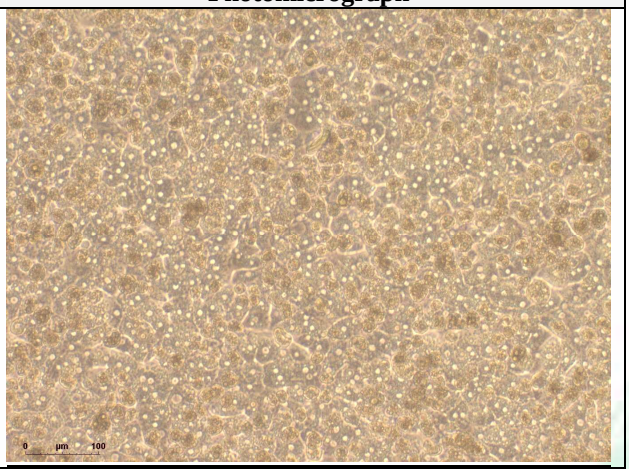
2 PRODUCT

CELL RECOVERY AND SEEDING			
Process		Human hepatocytes were isolated by standard 2-step methods.	
Day of cell recovery and seeding		February 03, 2015	
Cell seeding support (Rat collagen I coated plate/flask)		**Seeding density** ($x10^6$ cells/per support /well)	**Medium volume** (ml/per support/well)
96 well plate	☐	0.05	0.10
48 well plate	☒	0.17	0.22
24 well plate	☐	0.38	0.50
12 well plate	☐	0.76	1.0
6 well plate	☐	1.80	2.0
12.5 cm^2 Flask	☐	2.30	2.5
25 cm^2 Flask	☐	4.70	5.0
75 cm^2 Flask	☐	14.0	15.0

Cell culture period		**Basal medium**	**Additive**
22 hours in seeding medium	☒	William's E medium (MIL600***)	ADD221
day(s) in Short-Term medium (ST)*	☐	William's E medium (MIL600***)	ADD222

**Isolated and cultured hepatocytes", GUILLOUZO A. and GUGUEN-GUILLOUZO C., Eds INSERM Paris & John Libbey Eurotext London, 1986, pp.1-12.*
**** formerly MIL260*

3 CELL QUALITY CONTROL

Criteria	Specification	Accepted	Photomicrograph
Cell viability after isolation (D0)	≥ 80 %	Yes (82%)	
Cell culture confluence (D1)	≥ 90 %	Yes (100%)	
Cell morphology*	Ability to attach to collagen -coated support after overnight plating	Yes (see picture*)	Date of photomicrograph February 04, 2015 After 22 hours in the seeding medium

Sterility control	**Specification**	**Result**
Mycoplasma detection *(biochemical test)*	Negative	Negative ☒ Positive ☐ In progress ☐
Microbial sterility *(under standard use conditions)*	No microbial growth detectable	Undetectable ☒ Detectable ☐ In progress ☐

** Even if with a high initial % of viability, it is known that part of the hepatocytes isolated from a steatotic liver will die a few hours after seeding. We can observe that after a overnight culture period, some dead cells attach loosely to the top of the monolayer. Tapping the plate gently will dislodge these dead cells after receiving the plates and before changing culture medium.*

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4 CELL FUNCTIONAL CONTROL

Age of the culture at the time of controls: 1 day
(1 day after cell seeding)

Vmax value of Phase I dependent activities (nmole/h/million cells)

Activity	Enzyme	Result	Historical data					
			Minimum	1st quartile	Median	3rd quartile	Maximum	n
Phenacetin O-deethylase activity[(a)]	CYP1A2	**1.0**	0.7	2.1	3.3	5.1	18	139
Midazolam 1' hydroxylase activity[(a)]	CYP3A4/5	**0.6**	0.5	1.4	2.2	3.1	8.4	139
Bupropion hydroxylase activity[(a)]	CYP2B6	**0.9**	0.2	0.6	0.9	1.4	8.2	139
Dextrometorphan o demethylaseactivity [(a)]	CYP2D6	**0.4**	0.1	0.5	0.9	1.3	3.7	139
S-mephenytoin hydroxylase activity[(b)]	CYP2C19	**0.1**	<0.01	0.1	0.5	1.0	4.5	172

[(a)(b)] Cell monolayers were incubated either for 1 hour at 37°C with the following test substrates: phenacetin (200µM), midazolam (50µM), bupropion (100µM) and dextrometorphan (100µM) or for 5 hours with S-mephenytoin (200µM). Metabolites formed were measured by LC-MS/MS. Activities are expressed as nanomole of metabolite formed/h/million cells, which is equivalent to nmole/h/mg of protein. To convert to pmole of metabolite/min/million hepatocytes multiply the results by 16.66.

Vmax value of Phase II dependent activities (nmole/h/million cells)

Activity	Enzyme	Result	Historical data					
			Minimum	1st quartile	Median	3rd quartile	Maximum	n
Paracetamol glucuronidation activity	UGT1A1, UGT1A6, UGT1A9, UGT2B15,	**10**	3.1	8.6	11	15	130	304
Paracetamol sulfation activity	SULT1A1, SULT1A3/4, SULT1E1	**2.4**	1.6	3.9	5.1	6.4	100	301

Cell monolayers were incubated for 5 hours at 37°C with the paracetamol substrate (1mM). Metabolites formed were measured by LC-MS/MS. Activities are expressed as nanomole of metabolite formed/h/million cells, which is equivalent to nmole/h/mg of protein. To convert to pmole of metabolite/min/million hepatocytes multiply the results by 16.66.

5 CONDITIONS OF PRODUCT DELIVERY

Day of cell shipment	February 04, 2015		
Shipping medium	ST medium Phenol red-free ST medium	☒ ☐	
Shipping temperature	Room temperature	☒	
Product packaging	Use of BPI's patent-pending Sealovac™ Technology	☒	
	Use of adhesive film to seal culture plate	☐	
	Packaging warranty up to a maximum of	2 days ☒	3 days ☐
Protocol for use	Please follow the enclosed description and use protocol for human ST monolayer culture *"fdu_STHH_ protocol"*.		

6 VISA FOR BATCH RELEASE

Name	Signature	Date
Ruoya LI, Ph D		February 10, 2015

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