

SAMPLE

CERTIFICATE OF ANALYSIS
HUMAN CRYOPRESERVED HEPATOCYTES
GRADE P, QUALIFIED FOR PLATEABLE ASSAYS

Catalog number: PR-LIV-P
Batch number: HEP187730

FOR RESEARCH USE ONLY. Not intended for human or animal diagnostic or therapeutic uses. HUMAN CRYOPRESERVED HEPATOCYTES are not recommended for expanding. Human primary cells must be treated as potential pathogens. Users need to wear personal protective equipment during the work. DO NOT USE DRY ICE DURING WORK, STORAGE, OR TRANSPORTATION.

1. BIOLOGICAL MATERIAL
1.1. INFORMATION ABOUT DONOR

Age	71 years		
Sex	Male <input checked="" type="radio"/> Female <input type="radio"/>		
Ethnicity	Caucasian <input checked="" type="radio"/> African <input type="radio"/> Not available <input type="radio"/>		
Pathology or Cause of death	Hemorrhagic stroke		
Patient information	Diabetes	Yes <input type="radio"/>	No <input checked="" type="radio"/> Unknown <input type="radio"/>
	Heart disease	Yes <input checked="" type="radio"/>	No <input type="radio"/> Unknown <input type="radio"/>
	High blood pressure	Yes <input checked="" type="radio"/>	No <input type="radio"/> Unknown <input type="radio"/>
	Smoking	Yes <input type="radio"/>	No <input checked="" type="radio"/> Unknown <input type="radio"/>
	Alcoholism	Yes <input type="radio"/>	No <input checked="" type="radio"/> Unknown <input type="radio"/>
	Medication:	-	

Biological materials were collected from the certified clinical hospitals. Clinical site provided ethical committee approval and conducted the collection in accordance to the Directive 2004/23/EC of the European Parliament

1.2. SAFETY DATA

Virological status	Specification	Result
Hepatitis B (HBs antigen, anti HBc antibody)	Negative	Positive <input type="radio"/> Negative <input checked="" type="radio"/>
Hepatitis C (anti HCV antibody)		Positive <input type="radio"/> Negative <input checked="" type="radio"/>
HIV-1 and HIV-2 (anti HIV-1 and HIV-2 antibodies)		Positive <input type="radio"/> Negative <input checked="" type="radio"/>

1.3. LIVER TESTS

Name	Result	Reference
Alanine Aminotransferase (ALT)	37	4-42 U/l
Aspartate Aminotransferase (AST)	34	4-42 U/l
Gamma-Glutamyl Transferase (GGT)	74	5-61 U/l
Creatinine	98	48-108 uM/l

Total bilirubin	15.4	5-21 uM/l
Total protein	60	57-82 g/l

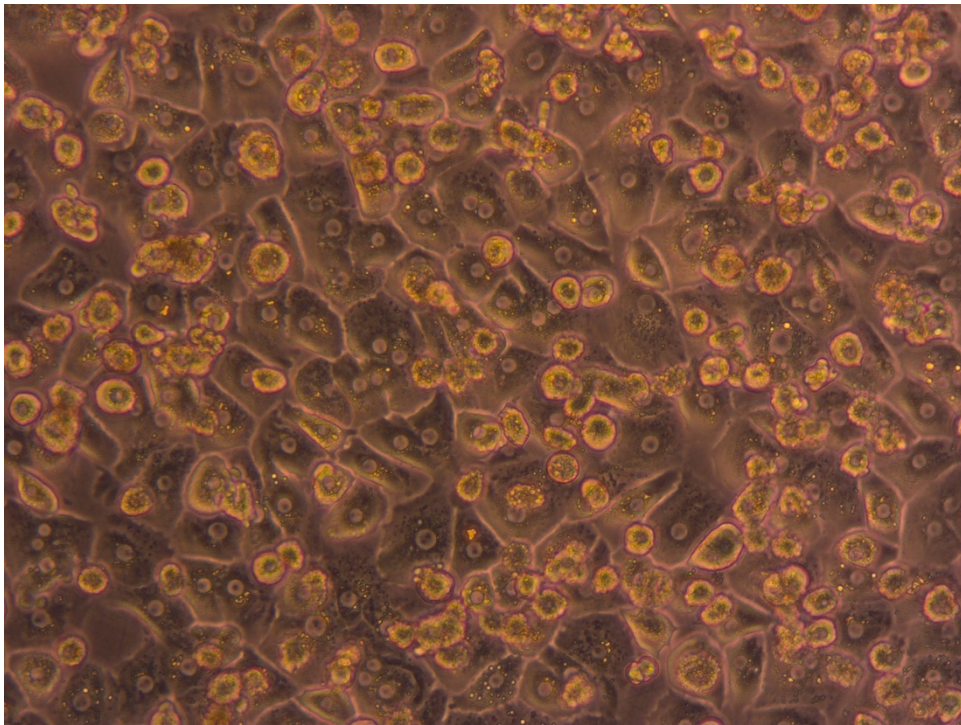
2. PRODUCT DESCRIPTION

Process	Human hepatocytes were isolated and frozen by standard methods.
Biosafety level	Human sourced products handled at the Biological Safety Level 2.
Date of production	15/11/2023
Cell quality control date	17/11/2023
Packaging	0.5 mL suspension in the cryovial with a minimum of 8x10 ⁶ viable cells.

3. CELL QUALITY CONTROL AFTER THAWING

Criteria	Specification	Result	CONCLUSION	
Post-thaw viability	≥ 80%	88 %	Yes ●	No ○
Number of viable cells per vial	≥ 8 x 10 ⁶	9,5 x 10 ⁶	Yes ●	No ○
Optimal Percoll concentration	25-28%	28 %	Yes ●	No ○
Cell confluence in 20-24 h of cultivation on collagen I coated plate	≥ 70%	85%	Yes ●	No ○
The ability of spheroids formation	Yes	Yes	Yes ●	No ○
Total protein test	250 µg/cm ²	300 µg/cm ²	Yes ●	No ○
Microbial sterility	No microbial growth detectable	Undetectable	Yes ●	No ○
Mycoplasma contamination	Standard test kit	Undetectable	Yes ●	No ○

4. MICROPHOTOGRAPH



24-hours of cultivation, 200x magnification

Magnification	x 200
Cell seeding density	0.5 mln cells per well
Well format	24-well tissue culture plate (IdMOC, Cat #71034)
Cultivation conditions	DMEM + 10% FBS + N21 supplement + 1% Penicillin/Streptomycin

5. METABOLIC ACTIVITY

Substrate	Intrinsic Clearance ($\mu\text{L}/\text{min}/10^6$ cells)	Enzymes Responsible for Metabolism
Dextromethorphan		CYP2D6 > 3A/2C19
Diclofenac		CYP2C9, UGT2B7, UGT1A9
Verapamil		CYP3A4
4-Hydroxycoumarin		Phase II
Propranolol		CYP2D6 >1A2/2C19/UGT

Imipramine		CYP2D6/1A2/2C19/3A/ UGT1A4
Raloxifene		CYP3A
Midazolam		CYP3A4
4-methylumbelliferone		UGT1A6/9
Tolbutamide		CYP2C
Ketoprofen		UGT2B7

6. CELL STORAGE

Delivery	In liquid nitrogen, $\leq 150^{\circ}\text{C}$
Storage temperature	In vapour of liquid nitrogen, $\leq 150^{\circ}\text{C}$ up to 5years

7. VISA FOR BATCH RELEASE

Name	Signature	Date
Tetiana Papurina	_____	17/11/2023

SAMPLE

Batch sheet revised on February 2022

CERTIFICATE OF ANALYSIS

20-DONOR MIXED GENDER POOLED CRYOPRESERVED HUMAN HEPATOCYTES

GRADE S, QUALIFIED FOR SUSPENSION ASSAYS

Batch number: **HEP190025-TA05**

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 DONOR DEMOGRAPHICS

Lot number	Gender	Ethnicity	Age	Tobacco Use	Alcohol Use	Serology testing (HIV/HBV/HCV)	Pathology
HEP187279	M	NA	82	No	NA	Negative	Liver metastasis
HEP187332	F	Caucasian	73	No	No	Negative	Liver metastasis (colon cancer)
HEP187369	M	Caucasian	48	Yes	NA	Negative	Liver metastasis
HEP187370	M	Caucasian	69	No	No	Negative	Bile duct cancer (cholangiocarcinomas)
HEP187436	M	Caucasian	67	No	No	Negative	Liver metastasis (colorectal cancer)
HEP187449	F	Caucasian	64	No	No	Negative	Huge Liver
HEP187533	F	NA	53	No	NA	Negative	NA
HEP187624	F	Caucasian	45	No	No	Negative	Hepatocellular Adenoma
HEP187647	F	Caucasian	57	No	NA	Negative	CRC with Liver Metastasis
HEP187701	F	Caucasian	64	No	No	Negative	Ca recti pT3pN1pM1
HEP187711	F	Caucasian	50	No	No	Negative	Ovarian cancer T1N0M0. Disease progression. Liver Mts
HEP187712	F	Caucasian	68	No	No	Negative	Colorectal cancer
HEP187714	F	Caucasian	48	No	No	Negative	Perihilar cholangiocarcinoma 3A
HEP187716	F	Caucasian	55	No	No	Negative	Klatskin tumor, Perihilar cholangiocarcinoma 4 type, T3NxM0
HEP187719	M	Caucasian	70	No	No	Negative	Colorectal liver metastasis
HEP187721	M	Caucasian	62	No	No	Negative	Colorectal cancer, liver metastasis
HEP187725	M	Caucasian	18	No	No	Negative	Left hepatectomy
HEP187726	M	Caucasian	59	No	No	Negative	Hemorrhagic stroke
HEP187727	M	Caucasian	63	NA	NA	Negative	Ca sigmoid with liver metastasis, pT3pN1M1
HEP187730	M	Caucasian	71	No	No	Negative	Hemorrhagic stroke

NA : Not Available. Additional donor's medication history is available upon request.

2 PRODUCT

DESCRIPTION	
Process	Human hepatocytes were isolated from liver resections and frozen by Biopredic's standard methods. The different batches of human hepatocytes were then pooled and frozen using a proprietary pooling process of TRL-Lonza under a license agreement.
Biosafety level	Human sourced products should be handled at the Biological Safety Level 2 (BSL 2)
Last Control Date	On January 24, 2024
Packaging	1 mL vial with a minimum of 5×10^6 viable cells
Quality Grade	Grade S qualified as non-plateable cryohepatocytes for suspension and metabolism assay

3 CONTROLS AFTER THAWING

Cell quality control

Criteria	Specification (One-step thawing)	Accepted Result ^a (One-step thawing)
Post-thaw viability	$\geq 85 \%$	Yes (88 %)
Number of viable cells per vial	$\geq 5 \times 10^6$	Yes (5.3×10^6)
Plateability hepatocytes ^b (post-thawing, in seeding medium)	Ability to attach to collagen-coated support after overnight plating	Un-plateable
Microbial growth (under standard use conditions)	No microbial growth detectable	Yes (Undetectable)

4 FUNCTIONAL CONTROLS AFTER THAWING

Controls performed on suspended cells

Clint value of the probe substrates for the Phase I enzymes ($\mu\text{L}\cdot\text{min}^{-1}\cdot\text{million cells}^{-1}$)

Substrate	Activity	Main enzyme involved	Result	Historic data					n
				Min	1st quartile	Med	3rd quartile	Max	
Phenacetin 1 μM	Phenacetin O-deethylation	CYP1A2	4.7	0.6	1.1	1.76	2.5	4.7	8
Coumarin 1 μM	Coumarin 7-hydroxylation	CYP2A6	49	15	24	28	43	49	8
Bupropion 1 μM	Bupropion hydroxylation	CYP2B6	1.8	0.3	0.4	0.49	0.8	1.8	8
Amodiaquine 1 μM	Amodiaquine N-deethylation	CYP2C8	642	36	81	95	118	642	8
Diclofenac 1 μM	Diclofenac 4'-hydroxylation	CYP2C9	11	4.9	5.8	7.5	9.6	11	8
Mephenytoin 5 μM	Mephenytoin hydroxylation	CYP2C19	0.08	0.0	0.02	0.04	0.07	0.08	8
Dextromethorphan 1 μM	Dextromethorphan O-demethylation	CYP2D6	11	2.2	3.9	5.6	7.6	11	8
Chlorzoxazone 1 μM	Chlorzoxazone 6-hydroxylation	CYP2E1	NA*	NA*					
Testosterone 5 μM	Testosterone 6 β -hydroxylation	CYP3A4/5	0.7	0.2	0.6	0.7	1.1	1.5	8
Nifedipine 1 μM	Nifedipine oxidation	CYP3A4/5	12	2.3	2.5	6.1	11	20	8

N/A : Not Available; U : Undetected

* Chlorzoxazone 6-hydroxylation activity isn't available because Chlorzoxazone's CY2E1 metabolite is directly taken up by phase II enzymes in human hepatocytes.

SAMPLE

5 CELL STORAGE. DELIVERY AND USE

Delivery and storage	In liquid nitrogen
Instruction for use of cells	Follow the description and use protocol for cryopreserved hepatocytes (fdu_Thawing Cryohep Opit ONE STEP V3 2018).

6 COMPANION PRODUCTS

CULTURE MEDIA		
Denomination	Use	Catalog number
Optimized thawing medium. 40mL for thawing 1 to 2 vials	Thawing medium	MIL130

7 VISA FOR BATCH RELEASE

Name	Signature	Date
Tetiana Papurina	_____	13/02/2024

CERTIFICATE OF ANALYSIS
HUMAN CRYOPRESERVED HEPATOCYTES
GRADE **SP**, QUALIFIED FOR **SPHEROID** ASSAYS

Catalog number: PR-LIV-SPH

Batch number: HEP187725

FOR RESEARCH USE ONLY. Not intended for human or animal diagnostic or therapeutic uses. HUMAN CRYOPRESERVED HEPATOCYTES are not recommended for expanding. Human primary cells must be treated as potential pathogens. Users need to wear personal protective equipment during the work. DO NOT USE DRY ICE DURING WORK, STORAGE, OR TRANSPORTATION.

1. BIOLOGICAL MATERIAL

1.1. INFORMATION ABOUT DONOR

Age	18 years		
Sex	Male <input checked="" type="radio"/> Female <input type="radio"/>		
Ethnicity	Caucasian <input checked="" type="radio"/> African <input type="radio"/> Not available <input type="radio"/>		
Pathology or Cause of death	Blunt head trauma		
Patient information	Diabetes	Yes <input type="radio"/>	No <input checked="" type="radio"/> Unknown <input type="radio"/>
	Heart disease	Yes <input type="radio"/>	No <input checked="" type="radio"/> Unknown <input type="radio"/>
	High blood pressure	Yes <input type="radio"/>	No <input checked="" type="radio"/> Unknown <input type="radio"/>
	Smoking	Yes <input type="radio"/>	No <input checked="" type="radio"/> Unknown <input type="radio"/>
	Alcoholism	Yes <input type="radio"/>	No <input checked="" type="radio"/> Unknown <input type="radio"/>
	Medication:	-	

Biological materials were collected from the certified clinical hospitals. Clinical site provided ethical committee approval and conducted the collection in accordance to the Directive 2004/23/EC of the European Parliament

1.2. SAFETY DATA

Virological status	Specification	Result
Hepatitis B (<i>HBs antigen, anti HBc antibody</i>)	Negative	Positive <input type="radio"/> Negative <input checked="" type="radio"/>
Hepatitis C (<i>anti HCV antibody</i>)		Positive <input type="radio"/> Negative <input checked="" type="radio"/>
HIV-1 and HIV-2 (<i>anti HIV-1 and HIV-2 antibodies</i>)		Positive <input type="radio"/> Negative <input checked="" type="radio"/>

2. PRODUCT DESCRIPTION

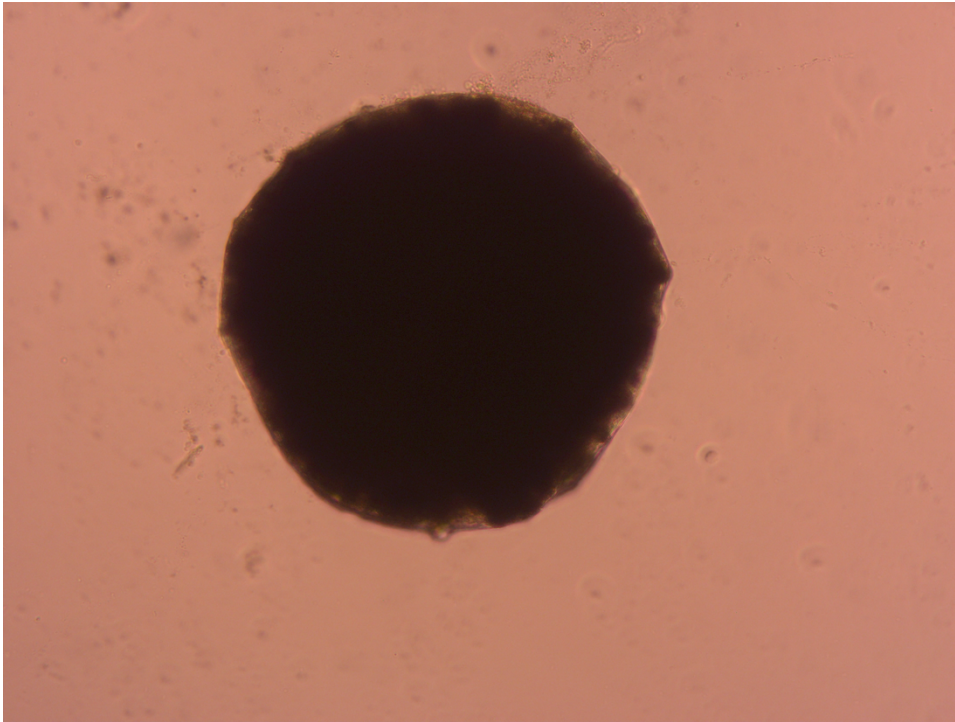
Process	Human hepatocytes were isolated and frozen by standard methods.
Biosafety level	Human sourced products handled at the Biological Safety Level 2.
Date of production	27/09/2023
Cell quality control date	29/09/2023
Packaging	0.5 mL suspension in the cryovial with a minimum of 1×10^6 viable cells.

3. CELL QUALITY CONTROL AFTER THAWING

Criteria	Specification	Result	Conclusion	
			Yes <input checked="" type="radio"/>	No <input type="radio"/>
Post-thaw viability	$\geq 80\%$	85 %	Yes <input checked="" type="radio"/>	No <input type="radio"/>
Number of viable cells per vial	$\geq 1 \times 10^6$	$1,7 \times 10^6$	Yes <input checked="" type="radio"/>	No <input type="radio"/>
The ability of spheroids formation	Yes	Yes	Yes <input checked="" type="radio"/>	No <input type="radio"/>
Number of days to form spheroids	7-14	10	Yes <input checked="" type="radio"/>	No <input type="radio"/>
Microbial sterility	No microbial growth detectable	Undetectable	Yes <input checked="" type="radio"/>	No <input type="radio"/>
Mycoplasma contamination	Standard test kit	Undetectable	Yes <input checked="" type="radio"/>	No <input type="radio"/>

4. MICROPHOTOGRAPHICS

SAMPLE




Magnification	x200
Cell seeding density	2000 cells/well
Well format	PhenoPlate™-384 ULA-coated (PerkinElmer, 6057800)
Cultivation conditions	DMEM + HepExtend supplement + 1% Penicillin/Streptomycin

5. CELL STORAGE

Delivery	In liquid nitrogen, $\leq 150^{\circ}\text{C}$
Storage temperature	In vapour of liquid nitrogen, $\leq 150^{\circ}\text{C}$ up to 5 years

6. VISA FOR BATCH RELEASE

Name	Signature	Date
Tetiana Papurina		10/10/2023