BIOPREDIC INTERNATIONAL

BIOLOGICALS FOR RESEARCH

HepaRG®
The hepatic stem cell line

CERTIFICATE OF ANALYSIS

DIFFERENTIATED HEPARG®-NS CRYOPRESERVED

Catalog number: HPR116NS

Batch number: HPR116NS080001

For in vitro and research use only

For more information, visit
www.HepaRG.com

LIMITED USE LICENSE

HepaRG® cells are patented and their use is strictly limited; consider the cells as a single-use, disposable product that must be destroyed upon conclusion of a study or experiment. Propagating, reproducing, cloning, subcloning or any other use of the cells following the conclusion of a study is prohibited. Use of the cells to produce or manufacture commercial products for general sale or for use in the manufacture of products intended for general sale is prohibited. Transfer of the cells to anyone not employed within the same organization, whether for financial benefit or not, is prohibited. **If you are unwilling to accept the terms of this LIMITED USE LICENSE, do not ORDER or use them, and immediately return the cells for credit. Violators of this Limited Use License will be prosecuted to the fullest extent of the law.**

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S.A.R.L. au capital de 98 500 euros • R.C.S. Rennes B - SIRET 391 701 232 00012 • APE 332B • VAT FR 21 391 701 232
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1. PRODUCT DESCRIPTION

Species	Human
Origin	Liver tumor of a patient suffering from hepatocarcinoma and hepatitis C infection
Safety data	Absence of hepatitis B, hepatitis C and HIV1 viruses checked by PCR on the cell suspension. *Caution: although controls are performed, human material has to be considered as potentially dangerous. Take maximum care in order to protect yourself and your colleagues.*
Biosafety level	Level 2, as recommended in the CDC-NIH Manual, Biosafety in Microbiological and Biomedical Laboratories,5th edition, N°21-1112 Revised December 2009
Description	HepaRG® cells, catalog number HPR116NS are cryopreserved after differentiation using a proprietary process developed by Biopredic International. HPR116NS is a new format of the HPR116 with a freezing process that allows direct thawing and seeding of cryopreserved differentiated cells without the need for post-thaw washing, centrifugation and counting steps. A few days after thaw and culture, the cells will form a coculture of hepatocytes and of biliary epithelial-like cells. Gripon P. *et al. Proc. Natl. Acad. Sci. 99, 15655-15660, 2002.*
Packaging	HPR116NS: 0.5 mL vial with ≥ 8 x 10^6 viable cells
CYP genotyping data	Obtained in collaboration with N. Picard at Limoges hospital (France) using a validated TaqMan allelic discrimination assay (ABI PRISM 7000 Sequence Detection System, Applied-Biosystems).

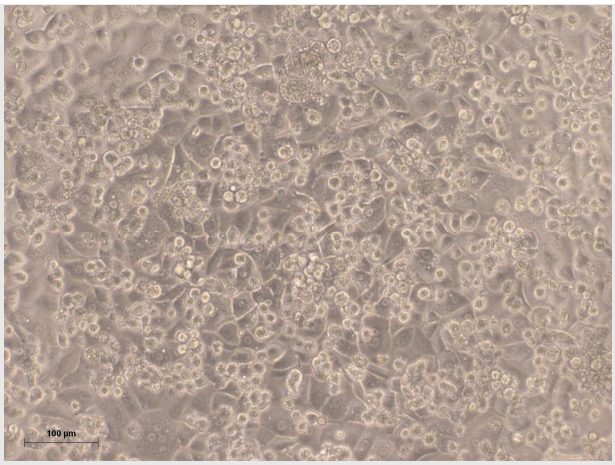
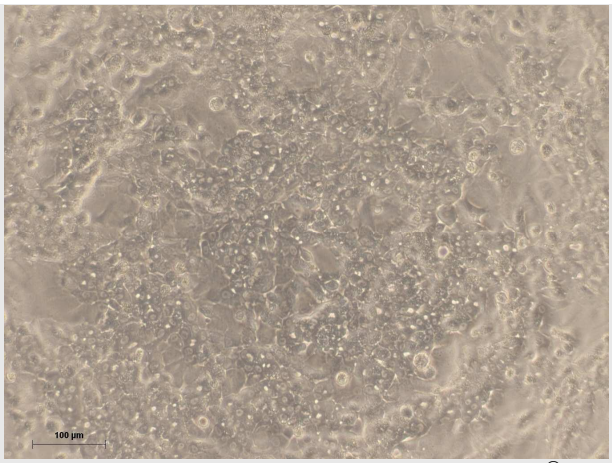
SNPs description SNPs: Single Nucleotide Polymorphisms wt: wild- type mut: mutation **: wt=G T=minor allele in Caucasians		
	CYP2D6	
	*2 (2850C>T)	*2/wt
	*3 (2549delA)	wt/wt
	*4 (1846G>A)	wt/wt
	*7 (2935A>C)	wt/wt
	*10 (100C>T)	wt/wt
	CYP3A4	
	*1B (392G>A)	wt/wt
	OATP1B1 (=OATP2)	
	*5 (521T>C)	wt/*5
	OATP1B3 (=OATP8)	
	334T>G	wt/wt**
	MRP2	
	-24C>T	wt/wt
	1249G>A	wt/mut
	3972C>T	wt/mut

2. PRODUCT CHARACTERIZATION

. Cell production

Cell freezing	On July 15, 2014
Passage number	Between 13 and 20

. Cell controls

Criteria	Method	Specification	Accepted
Number of viable cells per vial	Microscopic observation	≥ 8 x 10^6	Yes (9.7 x 10^6)
Post-thaw viability (Day 0) [a]	Trypan blue exclusion test	≥ 80 %	Yes (90 %)
Cell density [b]	Microscopic observation 6 hours after thawing and seeding cells	≥ 85 %	Yes (see picture)
		 **X 100 –** After thawing of cells and 6 hours of culture in the HepaRG®-NS Thawing and Plating medium using ADD411	
Cell morphology	Microscopic observation 72 hours after thawing and seeding cells, 50 % of typical hepatocyte-like cells are organized in clusters with formation of canaliculi-like structures.	 **X 100 –** After thawing of cells in the HepaRG®-NS Thawing and Plating medium using ADD411, and 48 hours in the HepaRG®-NS Pre-induction and Tox medium using ADD431	
Mycoplasma detection	Biochemical test	Negative	Yes (negative)
Microbial sterility	Under standard use conditions	No microbial growth detectable	Yes (undetectable)

[a]: After thawing, differentiated HepaRG® -NS viability is determined by Trypan blue (0.05% in PBS) exclusion test at 10 min post-thaw.

[b]: Differentiated HepaRG® -NS are seeded on collagen-coated multi-well culture plates in the HepaRG® -NS Thawing and Plating medium using ADD411, according to Biopredic's description and use guide for thawing, culture and use of cryopreserved differentiated HepaRG® -NS (fduHPR116NS).

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

Activity	Enzyme	Method	Specification	Result
Phenacetin O-deethylase activity	CYP1A2	After thawing of cells in the HepaRG®-NS Thawing and Plating medium using ADD411, cells were incubated in suspension in Invitrogen MEM medium # 51200 for 1 hour at 37°C with the following test substrates: - phenacetin (200µM), - midazolam (50µM), - bupropion (100µM), - dextrometorphan (100µM). Metabolites formed were measured by LC-MS/MS.	≥ 0.2	**0.4**
Midazolam 1' hydroxylase activity	CYP3A4/5		≥ 1	**2.5**
Bupropion hydroxylase activity	CYP2B6		≥ 0.05	**0.3**
Dextrometorphan O-demethylase activity	CYP2D6		≥ 0.05	**0.15**

Activities are expressed as nanomole of metabolite formed/h/million cells. To convert to pmole of metabolite/min/million cells multiply the results by 16.66.

. Induction of metabolic activity

Inducer	Enzyme	Assay	Method	Fold induction* activity of treated cells/activity of control cells	
				Specification	Result
Omeprazole, 50µM	CYP1A2	Phenacetin O-dethylation	After thawing of cells in the HepaRG®-NS Thawing and Plating medium using ADD411, and 48 hours in the HepaRG®-NS Pre-induction and Tox medium using ADD431, cells were incubated for again 48 hours in the HepaRG®-NS Serum Free Induction medium with corticoids using ADD451 with the test inducers. Cells were then incubated in Invitrogen MEM medium # 51200 with the test substrates : - for 2 hours with midazolam (50µM) - for 4 hours with bupropion (100µM), - for 5 hours with phenacetin (200µM). The formation of metabolites was measured by LC-MS/MS.	≥ 3.5	**3.6**
Phenobarbital, 1mM	CYP2B6	Bupropion-hydroxylation		≥ 3	**4**
Rifampicin, 10µM	CYP3A4/5	Midazolam 1'-hydroxylation		≥ 4	**8**

** The fold induction in CYP1A2, CYP2B6 and CYP3A4/5 activity was determined as a measure of specific metabolite formation relative to vehicle control cells (un-induced).*

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. Vmax value of uptake transporter activities (pmole/min/million cells)

Substrate	Transporter	Method	Specification	Result
[^{3}H] MPP+ 250µM	OCT	After thawing, cells were incubated in suspension at 37°C in uptake medium with either [^{3}H]MPP+, or [^{3}H]taurocholate for 3 minutes. The uptake transporter activity (Vmax) was measured as cell-associated radioactivity with a liquid scintillation counter and expressed in pmol/min/million cells. *Taurocholate is also a substrate of OATP.	≥ 50	**315**
[^{3}H] Taurocholate* 25µM	NTCP		≥ 6	**7.4**

3. CONDITIONS OF CELL SEEDING

With one vial of HPR116NS080001			
Cell culture support	**Cell seeding volume per well (mL)**	**Total volume of cell suspension (mL)**	**Maximum number of wells to be seeded**
6 well plate	2	**9.7**	**4**
12 well plate	1	**12.1**	**12**
24 well plate	0.5	**10.1**	**20**
48 well plate	0.2	**12.1**	**60**
96 well plate	0.1	**13.6**	**136**

Note that properly thawed cells will be in a suspension volume of 8mL so these figures are inclusive of that; for example when using 96 well plates with this Lot, 5.6 mL of working Thawing and Plating Media should be added to achieve a total volume of 13.6 mL.

4. CONDITIONS OF STORAGE AND DELIVERY

STABILITY, STORAGE, DELIVERY	
Stability and Storage	5 years in liquid nitrogen
Delivery	In dry ice or in liquid nitrogen If in dry ice, ensure shipment time did not exceed 48 hours
Use	Follow description and use guide for thawing, culture and use of cryopreserved differentiated HepaRG®-NS (fduHPR116NS).

5. COMPANION PRODUCTS

CULTURE MEDIA*	
Denomination	**Catalog number**
HepaRG®-NS Thawing and Plating Supplement with corticoids, to be combined with 100 mL of Basal medium w/o phenol red	ADD411
HepaRG®-NS Pre-induction and Tox Supplement with corticoids, to be combined with 100 mL of Basal medium w/o phenol red	ADD431
HepaRG® Serum-free Induction Supplement with corticoids, to be combined with 100 mL of Basal medium w/o phenol red	ADD451

**For details on use of supplements, consult the Description and Use Guide thawing, culture and use of cryopreserved differentiated HepaRG®-NS (fduHPR116NS)*

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CULTURE SUPPORT	
Denomination	**Catalog number**
6-well plate coated with collagen I*	PLA135
12-well plate coated with collagen I*	PLA138
24-well plate coated with collagen I*	PLA137
48-well plate coated with collagen I*	PLA139
96-well plate coated with collagen I*	PLA136

**BPI proprietary coating process to ensure proper seeding and culture of the HPR116*

6. OTHER OFFERING OF THE HEPARG® CELLS

Denomination	**Catalog number**
"Grow and use" sublicense for in-house production of HepaRG®. Labs can acquire undifferentiated, cryopreserved HepaRG® cells for on-site propagation and differentiation, by signing a sublicense contract with Biopredic International. Please inquire about our Biopredic International's conditions.	HPR101

7. VISA BATCH RELEASE

Name	**Signature**	**Date**
Sandrine Camus, MSc		On August 08, 2014