BIOPREDIC INTERNATIONAL

BIOLOGICALS FOR RESEARCH

HepaRG®
The hepatic stem cell line

CERTIFICATE OF ANALYSIS
DIFFERENTIATED HEPARG®-NS CRYOPRESERVED
Catalog number: HPR116NS

Batch number: HNS1003

For in vitro and research use only

For more information, visit
www.HepaRG.com

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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

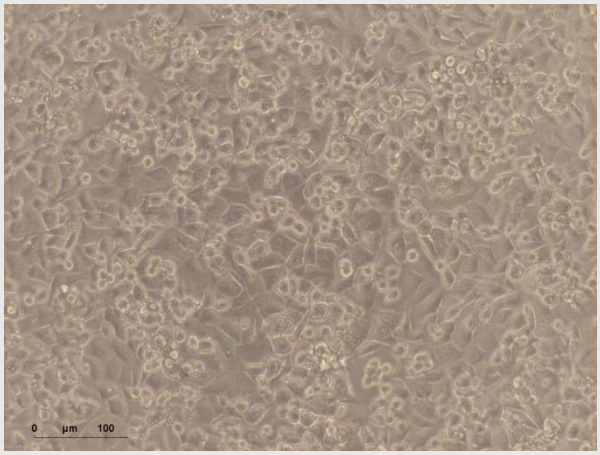
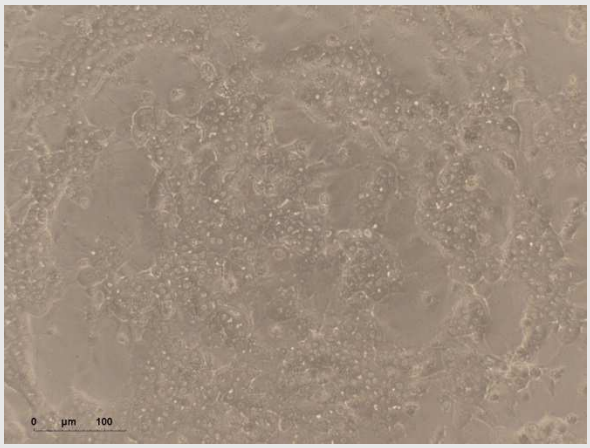
Species	Human	
Origin	Liver tumor of a patient suffering from hepatocarcinoma and hepatitis C infection	
Safety data	Absence of hepatitis B, hepatitis C and HIV1 viruses checked by PCR on the cell suspension. *Caution: although controls are performed, human material has to be considered as potentially 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	**CYP2D6**	
	*2 (2850C>T)	*2/wt
	*3 (2549delA)	wt/wt
	*4 (1846G>A)	wt/wt
mut: mutation **: wt=G T=minor allele in Caucasians	*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 December 10, 2015
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 (10 x 10^6)
Post-thaw viability (Day 0) [a]	Trypan blue exclusion test	≥ 80 %	Yes (94 %)
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/Plating medium using ADD411	
Cell morphology	Microscopic observation 4 days after thawing and seeding cells, 50 % of typical hepatocyte-like cells are organized in well-delineated clusters with bright canaliculi-like structures.	 **X 100 –** After thawing of cells in the HepaRG®-NS Thawing/Plating medium using ADD411, and 72 hours in the HepaRG®-NS Maintenance/Metabolism Medium using ADD421	
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/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/Plating medium using ADD411, cells were incubated in suspension 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	**0.3**
Midazolam 1' hydroxylase activity	CYP3A4/5		≥ 1	**2.2**
Bupropion hydroxylase activity	CYP2B6		≥ 0.05	**0.2**
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/Plating medium using ADD411, and 48 hours in the HepaRG®-NS Pre-induction/Tox medium using ADD431, cells were incubated for again 48 hours in the HepaRG®-NS Serum Free Induction medium using ADD451 with the test inducers. Cells were then incubated 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	**6**
Phenobarbital, 1mM	CYP2B6	Bupropion-hydroxylation		≥ 3	**6**
Rifampicin, 10µM	CYP3A4/5	Midazolam 1'-hydroxylation		≥ 4	**19**

** 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).*

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

Substrate	Transporter	Method	Mean value +/- confidence interval of 90%**
[^{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.	**307 +/- 23**
[^{3}H] Taurocholate* 25µM	NTCP		**12 +/- 0.9**

*** Obtained from 30 batches tested*

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3. CONDITIONS OF CELL SEEDING

With one vial of HPR116NS batch HNS1003			
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	**10**	**5**
12 well plate	1	**12.5**	**12**
24 well plate	0.5	**10.4**	**20**
48 well plate	0.2	**12.5**	**62**
96 well plate	0.1	**13.9**	**138**

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, 5.9 mL of working Thawing/Plating Media should be added to achieve a total volume of 13.9 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/Plating Medium Supplement with corticoids, to be combined with 100 mL of Basal medium w/o phenol red	ADD411
HepaRG™-NS Maintenance/Metabolism Medium Supplement with corticoids, to be combined with 100 mL of Basal medium w/o phenol red	ADD421
HepaRG®-NS Pre-induction/Tox Medium Supplement with corticoids, to be combined with 100 mL of Basal medium w/o phenol red	ADD431
HepaRG® Serum-free Induction Medium 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)*

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*

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6. OTHER OFFERING OF THE HEPARG® CELLS

Denomination	Catalog number
Undifferentiated, cryopreserved HepaRG® cells for on-site propagation and differentiation. Please see on our HepaRG® web site www.heparg.com for the conditions to obtain the cell line.	HPR101
HepaRG® differentiated cells, cryopreserved after differentiation using a proprietary process developed by Biopredic International.	HPR116
HepaRG® undifferentiated cells, cryopreserved with a sufficient quantity of culture medium for production of 50 plates within a 6 month period of time.	KIT901

7. VISA BATCH RELEASE

Name	Signature	Date
Sandrine Camus, Product Chief		On January 29, 2016
Belkacem Bouaita, Quality Control Manager		On January 29, 2016

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