

The hepatic stem cell line

CERTIFICATE OF ANALYSIS

DIFFERENTIATED HEPARG[®]-NS CRYOPRESERVED

Catalog number: HPR116NS

Batch number: HNS1002

For in vitro and research use only

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www.HepaRG.com

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1. PRODUCT DESCRIPTION

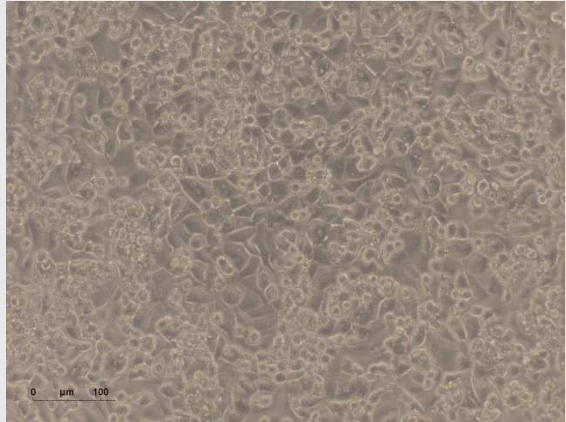
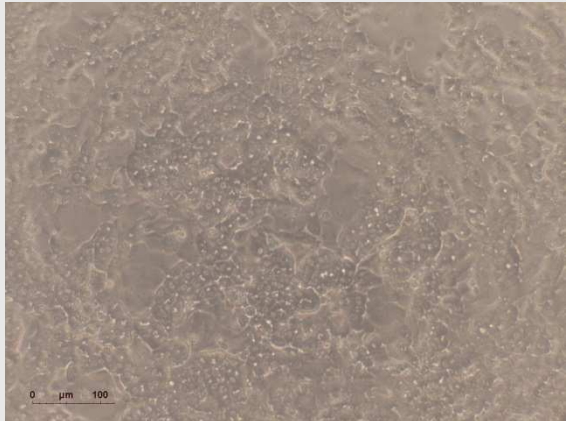
| | | |
|---|--|---------|
| Species | Human | |
| Origin | Liver tumor of a patient suffering from hepatocarcinoma and hepatitis C infection | |
| Safety data | Absence of hepatitis B, hepatitis C and HIV1 viruses checked by PCR on the cell suspension. <i>Caution: although controls are performed, human material has to be considered as potentially dangerous. Take maximum care in order to protect yourself and your colleagues.</i> | |
| 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. <i>et al. Proc. Natl. Acad. Sci. 99, 15655-15660, 2002.</i> | |
| Packaging | HPR116NS: 0.5 mL vial with $\geq 8 \times 10^6$ 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 | CYP2D6 | |
| SNPs: Single Nucleotide Polymorphisms wt: wild- type | *2 (2850C>T) | *2/wt |
| | *3 (2549delA) | wt/wt |
| mut: mutation **: wt=G T=minor allele in Caucasians | *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 October 15, 2015 |
| Passage number | Between 13 and 20 |

. Cell controls

| Criteria | Method | Specification | Accepted |
|--|---|--|---------------------------|
| Number of viable cells per vial | Microscopic observation | $\geq 8 \times 10^6$ | Yes (8.1×10^6) |
| Post-thaw viability (Day 0)^a | Trypan blue exclusion test | $\geq 80 \%$ | Yes (94 %) |
| Cell density^b | Microscopic observation 6 hours after thawing and seeding cells | $\geq 85 \%$ | Yes (see picture) |
| | |  <p>X 100 – After thawing of cells and 6 hours of culture in the HepaRG[®]-NS Thawing/Plating medium using ADD411</p> | |
| 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. |  <p>X 100 – After thawing of cells in the HepaRG[®]-NS Thawing/Plating medium using ADD411, and 48 hours in the HepaRG[®]-NS Maintenance/Metabolism Medium using ADD421</p> | |
| 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).

. 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 |
| Bupropion hydroxylase activity | CYP2B6 | | ≥ 0.05 | 0.2 |
| Dextrometorphan O-demethylase activity | CYP2D6 | | ≥ 0.05 | 0.2 |

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 | 5 |
| Phenobarbital, 1mM | CYP2B6 | Bupropion-hydroxylation | | ≥ 3 | 6 |
| 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).

. 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 | 259 |
| [³ H] Taurocholate* 25µM | NTCP | | ≥ 6 | 14 |

3. CONDITIONS OF CELL SEEDING

With one vial of HPR116NS batch HNS1002

| 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 | 8.1 | 4 |
| 12 well plate | 1 | 10.1 | 10 |
| 24 well plate | 0.5 | 8.4 | 16 |
| 48 well plate | 0.2 | 10.1 | 50 |
| 96 well plate | 0.1 | 11.2 | 112 |

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, 3.2 mL of working Thawing/Plating Media should be added to achieve a total volume of 11.2 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*

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 November 20, 2015 |
| Belkacem Bouaita, Quality Control Manager |  | On November 20, 2015 |