

ANALYSIS CERTIFICATE

Lot#: BHum16031

PRODUCT DESCRIPTION

Reference: HuMC

Product: Human Hepatocytes

Category: Cryopreserved Plateable

Isolation date: 15 March 2016

Initial Isolation Viability (%): 82%

Storage conditions: Storage conditions: -196°C

DONOR DEMOGRAPHICS

Species	Sex	Race	Age	BMI	Smoker	Alcohol Use	Drug Use
Human	Male	Caucasian	67	N/A	No	No	No

Pathology	Medication	Serological Data
Neo colon hepatic metastases	Omeprazole, Omnic	Negative tested less than 3 months before operation

Patient informed consent was obtained. The donor was serologically tested negative for following infectious diseases: HIV, Hepatitis B and C. Donor medical history were also examined prior to accepting this donor.

For in vitro use only, not to be used for clinical application. Products distributed by Cytes Biotechnologies may contain human material that should be treated as potentially hazardous.

CHARACTERIZATION FOR SUSPENSION CELLS

Lot#: BHum16031

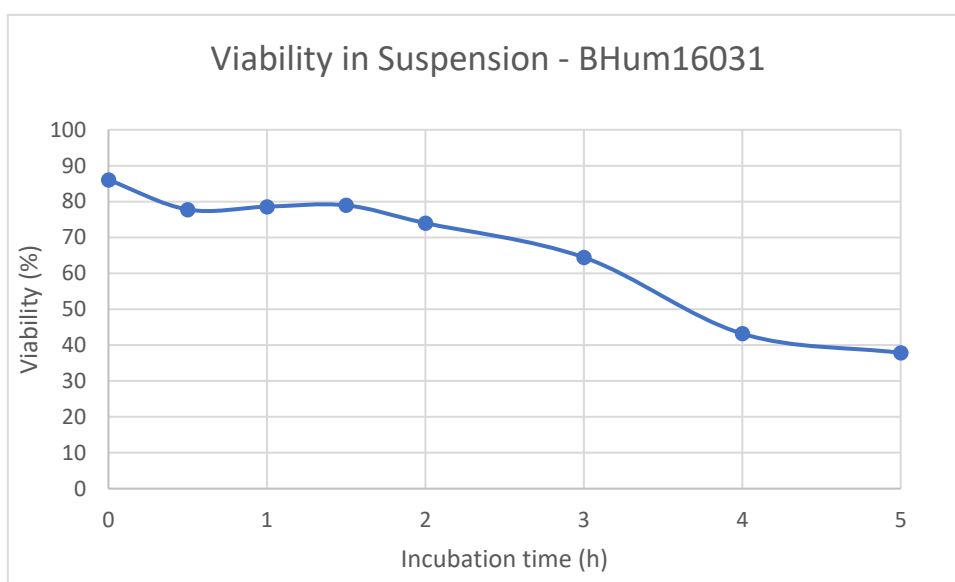
Post Thaw Lot information

Number of viable cells/vial: $5.0 \pm 2.4 \times 10^6$

Post-thaw viability (%): 87 ± 1.4

Human hepatocytes were thawed according to Cytes Biotechnologies protocol. The post-thawing yield and viability (trypan blue exclusion assay) of hepatocytes were assessed after a purification process.

Time (h)	0	0.5	1	1.5	2	3	4	5
Viability (%)	86.0	77.8	78.6	78.9	74.0	64.4	43.2	37.8



Resuspended human hepatocytes suspension ($0,5 * 10^6$ cells in 0.5 ml medium) from the post-thaw assessment were incubated up to 5 h at 37°C. The assay was performed in 2 ml round-bottom tubes under shaking conditions (1000 rpm) using Eppendorf Thermomixer C. In the first two hours, samples were taken at every 30 min, after 2 h samples were taken at every 60 min. At each time point the viability of cells was calculated

ENZYME ACTIVITY IN SUSPENSION CELLS

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- **PHASE I: CYP ACTIVITIES EXPRESSED IN pmol/min/mg protein (mean ± SD)**

Enzyme Activity	
Isoforms	Activity (pmol/min/mg protein)
CYP1A2	38.04 ± 0.95
CYP2B6	39.77 ± 0.07
CYP3A4/5	1.11 ± 0.09

Cryopreserved human hepatocytes in suspension culture (0.5×10^6 cells in 0.5 ml medium) were incubated with specific substrates (see table below) for 30 min at 37 °C for determination of CYP activities. The assay was performed in 2 ml round-bottom tubes under shaking conditions (1.000 rpm) in Eppendorf Thermomixer C. Metabolites were quantified by LC-MS and normalized to protein content. The substrates were applied as cocktail for simultaneous assessment of CYP 450 activity. Results are expressed in pmol/mg*min.

Table 1. Substrates Phase I

Enzyme	Probe Substrate	Concentration (μ M)	Incubation Time (min)	Metabolite
CYP1A2	Phenacetin	100	30	Acetaminophen
CYP2B6	Bupropion	500	30	Hydroxybupropion
CYP3A4/5	Midazolam	3	30	1-Hydroxymidazolam

Signed:



Quality Manager
Unit for Research
Cytes Biotechnologies

