

# CERTIFICATE OF ANALYSIS

Page 1 of 3

## Cell Line Details

**Product code:** HEP-003-ATP7B-b

**Product name:** iPSC-derived Human Hepatocytes: CRISPR-engineered Homozygous Wilson's Disease H1069Q

**Lot number:** XXXXXX

**Storage conditions:** Store at less than -130°C

**QC completion date:** XXXXXX

## Cell Quality Controls

Test	Method	Specification	Result
Virus test for original iPSC clone (HIV1, HIV2, Hepatitis A, HBV, HCV, HTLV-1, HTLV-2)	PCR	Not detected	Pass
Post thaw viability	Automated cell counter	≥ 70% viable	Pass
Viable cells per vial	Automated cell counter	≥ 5.0 x 10 <sup>6</sup>	Pass
Cell morphology	Visual check	N/A	Pass (Fig.1)
Key hepatocyte maturity markers ( <i>ALB</i> , <i>A1AT</i> , <i>HNF4a</i> )	qPCR	Present	Pass (Fig.2)
Detection of <i>ATP7B</i> via qPCR	qPCR	Present	Pass (Fig.3)
Disease confirmation	Sanger sequencing	Mutation present	Pass (Fig.4)

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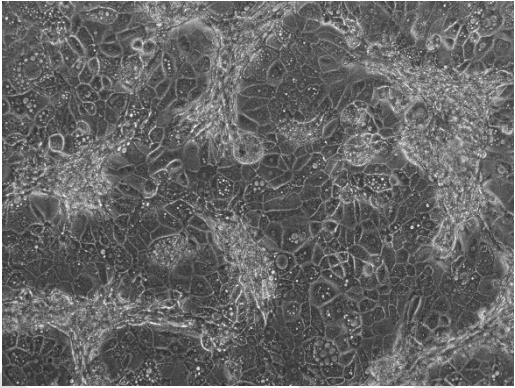
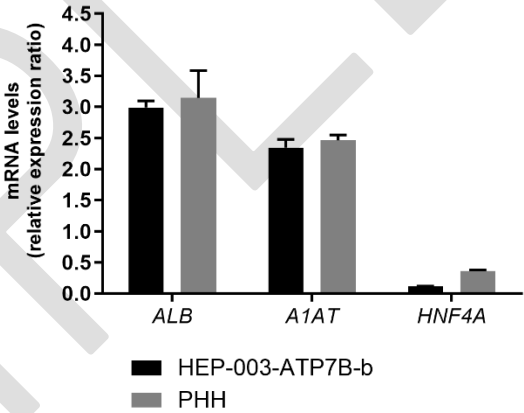
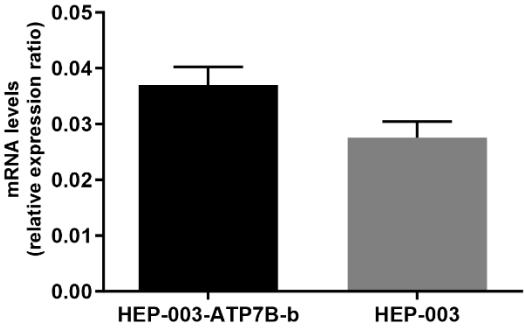
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Page 2 of 3

## Appendix

<p><b>1. Cell morphology</b></p> <p><b>Figure 1.</b> Morphology of cryopreserved hepatocyte-like cells, 14 days post-thaw. Brightfield picture, magnification: 100x.</p>													
<p><b>2. Detection of hepatocyte maturity markers via qPCR</b></p> <p><b>Figure 2.</b> mRNA expression of the key hepatocyte maturity markers <i>ALB</i> (Albumin), <i>A1AT</i> (Alpha-1 Antitrypsin) and <i>HNF4A</i> (Hepatocyte Nuclear Factor-4) in cryopreserved hepatocyte-like cells (black bars) and primary human hepatocytes (PHH, grey bars), 14 days post-thaw. mRNA data are normalized to endogenous <i>PPIA</i> expression, and are presented as mean±SD of n=3 technical replicates.</p>	 <table border="1"> <caption>mRNA levels (relative expression ratio)</caption> <thead> <tr> <th>Marker</th> <th>HEP-003-ATP7B-b</th> <th>PHH</th> </tr> </thead> <tbody> <tr> <td>ALB</td> <td>~3.0</td> <td>~3.2</td> </tr> <tr> <td>A1AT</td> <td>~2.4</td> <td>~2.5</td> </tr> <tr> <td>HNF4A</td> <td>~0.1</td> <td>~0.4</td> </tr> </tbody> </table>	Marker	HEP-003-ATP7B-b	PHH	ALB	~3.0	~3.2	A1AT	~2.4	~2.5	HNF4A	~0.1	~0.4
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<p><b>3. Detection of ATP7B via qPCR</b></p> <p><b>Figure 3.</b> mRNA expression of <i>ATP7B</i> (ATPase Copper Transporting Beta) in cryopreserved hepatocyte-like cells (black bar) and their isogenic control (grey bar), 14 days post-thaw. mRNA data are normalized to endogenous <i>PPIA</i> expression, and are presented as mean±SD of n=3 technical replicates.</p>	 <table border="1"> <caption>mRNA levels (relative expression ratio)</caption> <thead> <tr> <th>Cell Type</th> <th>mRNA levels (relative expression ratio)</th> </tr> </thead> <tbody> <tr> <td>HEP-003-ATP7B-b</td> <td>~0.037</td> </tr> <tr> <td>HEP-003</td> <td>~0.027</td> </tr> </tbody> </table>	Cell Type	mRNA levels (relative expression ratio)	HEP-003-ATP7B-b	~0.037	HEP-003	~0.027						
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Page 3 of 3

## 4. Disease confirmation via Sanger sequencing

Ref seq **CTGTGGTGGGGACTGCGGAGGCCAGCAGTGAACACCCCTTGGGCGTGGCAGTACCA**  
HEP-003-ATP7B-b **CTGTGGTGGGGACTGCGGAGGCCAGCAGTGAACAA**CCCTTGGGCGTGGCAGTACCA

**Figure 4.** Sanger sequencing showing homozygous missense mutation H1069Q (CAC>CAA) in the *ATP7B* gene. The codon change is highlighted in yellow. The wild type sequence (Ref Seq) is shown at the top while the mutant line at the bottom.

Checked by,

*signature*

QC Scientist

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