# CERTIFICATE OF ANALYSIS



DefiniGEN
DISEASE MODEL INNOVATION

## Cell Line Details

**Product code:** HEP-003-AAT-z

**Product name:** iPSC-derived Human Hepatocytes: CRISPR-engineered Homozygous Alpha-1 Antitrypsin Deficiency E342K

**Lot number:** XXXXXX

**Storage conditions:** Store at less than -130ºC

**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 | ≥ $5.0 \times 10^6$ | Pass |
| Cell morphology | Visual check | N/A | Pass (Fig.1) |
| Key hepatocyte maturity markers (*ALB, A1AT, HNF4a)* | qPCR | Present | Pass (Fig.2) |
| Disease markers (intracellular polymeric A1AT) | ELISA | ≥ 3.0 (fold change over WT) | Pass (Fig.3) |
| Disease confirmation | Sanger sequencing | Mutation present | Pass (Fig.4) |



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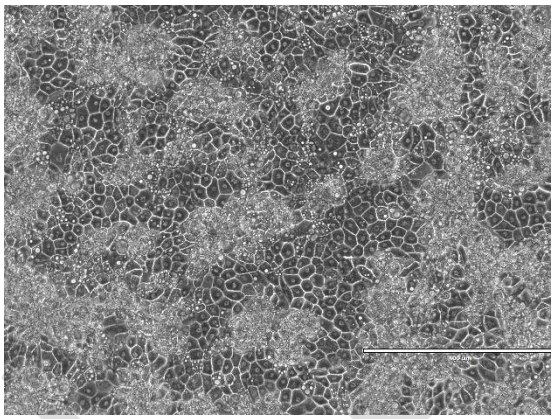
## Appendix

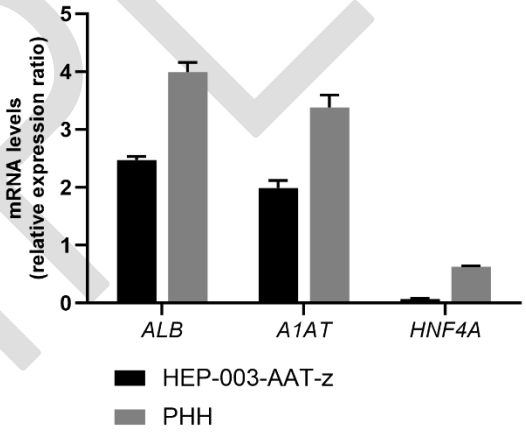
### 1. Cell morphology

**Figure 1.** Morphology of cryopreserved hepatocyte-like cells, 14 days post-thaw. Brightfield picture, magnification: 100x.



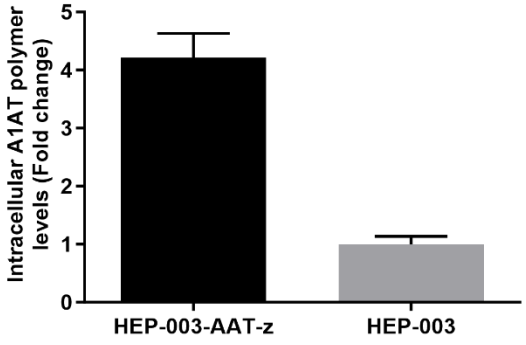
### 2. Detection of hepatocyte maturity markers via qPCR

**Figure 2.** mRNA expression of the key hepatocyte maturity markers *ALB* (Albumin), *A1AT* (Alpha-1 Antitrypsin) and *HNF4A* (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 *PPIA* expression, and are presented as mean±SD of n=3 technical replicates.




### 3. Detection of intracellular A1AT polymers via ELISA

**Figure 3.** Intracellular polymers detected by 2C1 ELISA 14 days post-thaw. The graph shows relative values, calculated by normalizing A1AT polymer content (ng/ml) to total protein in each cell lysate. Data normalized to wild type isogenic control (HEP-003), set as 1.



## 4. Disease confirmation via Sanger sequencing

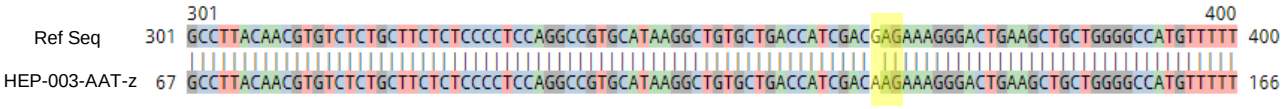



**Figure 4.** Sanger sequencing showing homozygous E342K mutation (GAG > AAG) in the *SERPINA1* gene. The codon change is highlighted in yellow. The wild type sequence (Ref Seq) is shown at the top while the mutant line at the bottom.

Checked by,

*signature*

QC Scientist



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