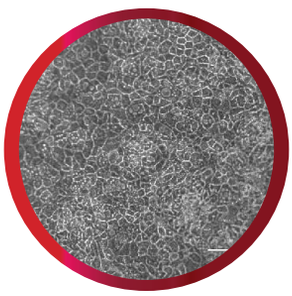
DefiniGEN

# DefiniGEN human iPS-derived A1ATD hepatocytes



Disease modelled alpha-1 antitrypsin deficiency hepatocytes (Def-HEP A1ATD) are highly functional patient-derived human hepatocytes. The cells are generated using dermal fibroblasts from an alpha-1 antitrypsin (A1AT) diseased patient carrying the 'Z' (E342K) point mutation in the A1AT gene SERPINA1. This mutation leads to the formation of mutant A1AT polymers that ultimately cause liver and lung damage.

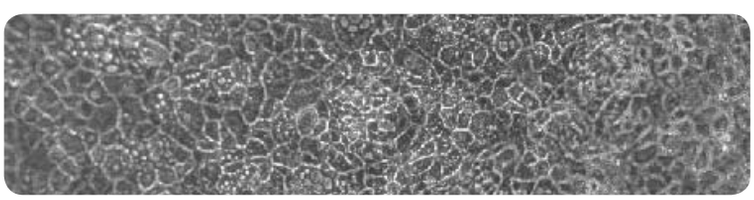


**Figure 1.** Clear cobblestone morphology of Def-HEP A1ATD cells post-thaw. Cell seeding: $0.5x10^6$ cells/well in collagen type 1 coated 24 well plate. Confluent monolayer. Magnification level: x10.

## General hepatocyte maturation markers

Def-HEP A1ATD are functionally mature human hepatocyte cells. Therefore in addition to their specific disease circuit they display general hepatocyte maturation markers such as albumin, A1AT, CK18 and HNF4a (Figure 2).

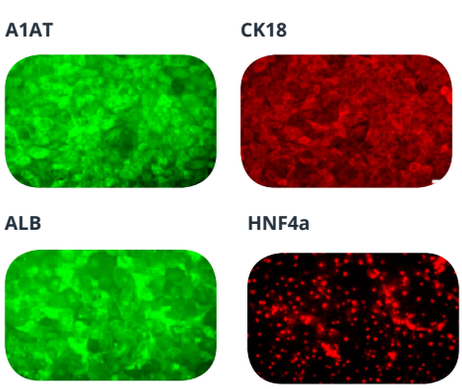



**Figure 2.** Immunostaining of Def-HEP A1ATD cells overviewing the expression of general hepatocyte maturation markers.

## Advantages

**Standardized cell product** containing >98% human hepatocyte cells producing reproducible and biologically relevant data

**Normal human genetics** patient donor genetics and karotype verified

**Disease circuit verification** ZZ mutation in SERPINA1 gene leads to build up of mutant A1AT polymer in the cells

**Optimized work flow** we can deliver industrial quantities of cryopreserved cells to client specification

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For the production of the Def-HEP A1ATD cells, A1ATD patient fibroblasts are first reprogrammed into iPSC using the Nobel Prize winning technology developed by Yamanaka and colleagues. These iPSC are then differentiated into liver hepatocytes using the OptiDIFF protocol developed at the University of Cambridge Laboratory for Regenerative Medicine. Def-HEP A1ATD patient derived hepatocytes represent an optimized disease model for drug discovery applications and are an effective tool for elucidating the underlying mechanisms of the disease.

## Detection of disease markers via ELISA

The A1AT disease marker can be quantified using an ELISa for mutant polymer and wild-type secreted A1AT. The assay utilizes antibodies specific for A1AT polymers (2C1mAb, top) or all conformers of A1AT (bottom) (Figure 3).

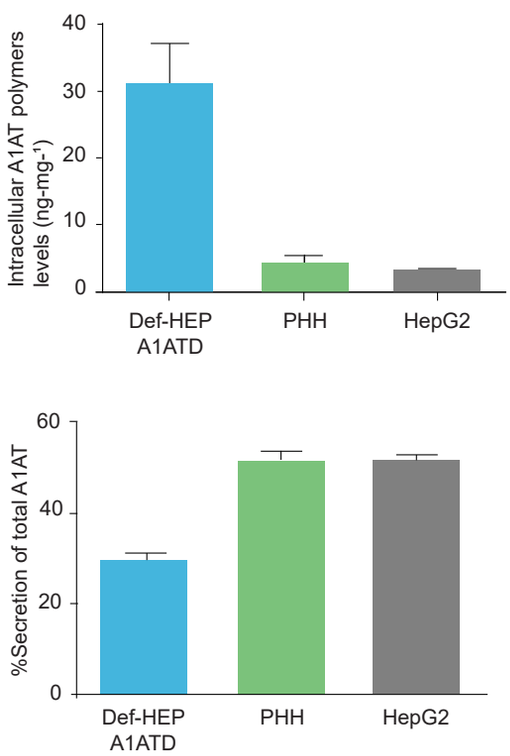


**Figure 3.** Quantification of intracellular A1AT polymers and intercellular total A1AT secretion in Def-HEP A1ATD cells. Primary human hepatocytes (PHH) and hepatocellular liver carcinoma cells (Hep G2) are used as internal controls.

## Immunocytochemistry analysis of A1AT mutant polymer

Previous studies have shown that the Z allele (Glu342Lys) results in the formation of ordered polymers of a1-antitrypsin that are retained within the ER. This pathway of a1-antitrypsin polymerization is central to the clinical phenotype. We therefore used the 2C1 polymer specific monoclonal antibody to detect polymers within Def-HEP A1ATD hepatocytes. Polymers were detected by immunostaining (Figure 4). The immunocytochemistry data anlysis show that accumulation of a1-antitrypsin polymers only occurs in disease-specific human Def-HEP hepatocytes from individuals with A1ATD; no polymers are present in human iPS cell-derived hepatocytes from control subjects.

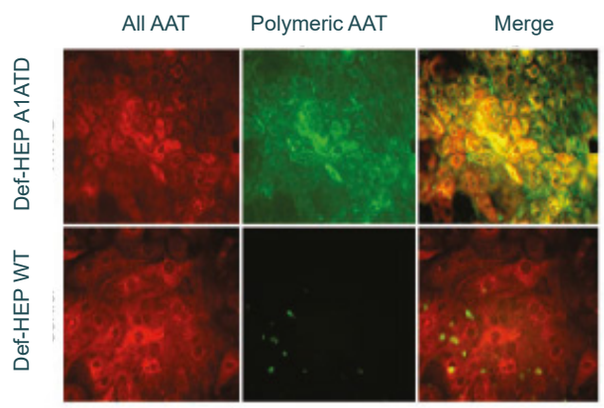


**Figure 4.** Immunostaining analyses for expression of misfolded polymeric a1-antitrypsin using the polymer- specific 2C1 antibody (green) or an antibody that detects all forms a1-antitrypsin (red) in Def-HEP A1ATD disease modelled cells and control human iPS cell-derived hepatocytes. Merged images are shown at right.

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