

# KC-2121446

**Supplier:** LifeNet Health

**Test date:** November 2022

**Experiment:** CLT22009

## Overview

This report describes the validation of primary human Kupffer cells (KC) co-cultured with primary human hepatocytes (PHHs) for liver-on-a-chip assays.

Our quality control procedure ensures that donor cell combinations form stable three-dimensional liver microtissues that maintain a hepatic phenotype and responded to immunomodulator (LPS) treatment when cultured over 5 days by PhysioMimix<sup>®</sup> OOC Systems.

## Protocol

Duo-cultures of a previously qualified PHH donor (see sample Primary human hepatocyte validation report for more info) and KCs were seeded into a PhysioMimix Multi-chip Liver-12 plate on day 0 at  $4 \times 10^5$  and  $4 \times 10^4$  cells per well, respectively.

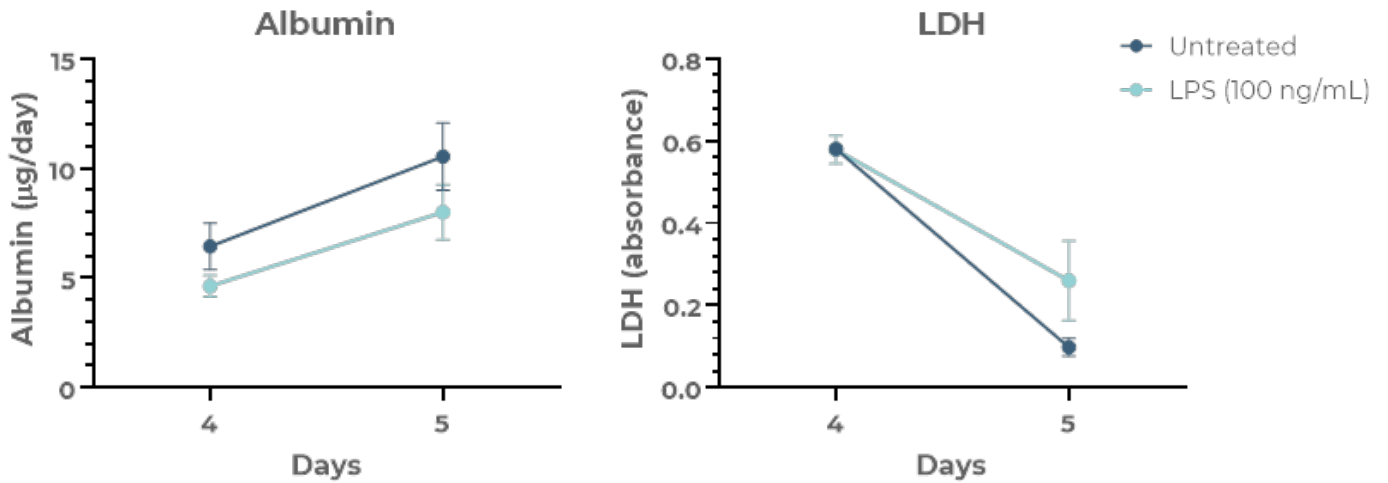
To assess cell health throughout the experiment, LDH and albumin were measured at each media change (days 4 and 5. Fig 1).

Cells were treated with 100 ng/mL LPS or left untreated on day 4. IL-6 and TNF $\alpha$  inflammatory markers, were measured on days 4 and 5 to assess the KC inflammatory response (Fig 2).

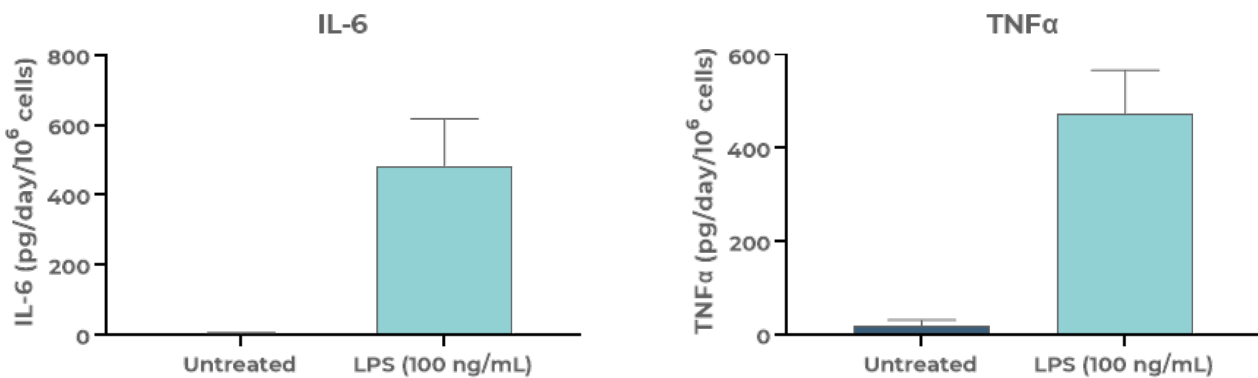
On day 5 the scaffolds within each well of the Multi-chip Liver-12 plate were removed, and the microtissues within the scaffold's pores were fixed and imaged using a brightfield microscope (10x magnification) to assess the quality of tissue formation (Fig 3). The qualification followed our 001153\_Rev01 KC Validation Protocol.

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## Validation Data



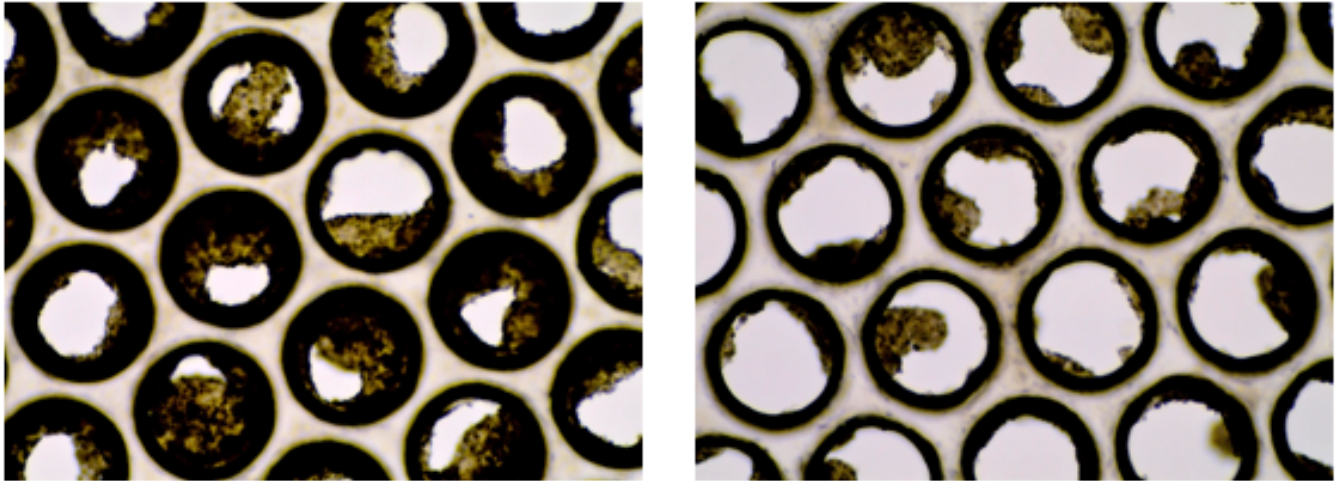
**Figure 1 – Cell health analysis.** Cell health analysis. Quantification of LDH and albumin levels within media samples at days 4 and 5 of the culture by colorimetric assay or ELISA, respectively.”



**Figure 2 – Cell inflammatory response analysis.** Quantification of TNF $\alpha$  and IL6 within media samples taken at day 5 of the culture as measured by ELISA. Data presented as mean with SD from triplicate samples.

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### Microtissue formation



**Figure 3 – Brightfield Microscopy.** Representative image taken at 10X from one scaffold removed from the Liver-12 plate at day 5.