

KC-287345

Supplier: LifeNet Health

Test date: August 2024

Experiment: DIAB_08122024

Overview

This report describes the validation of primary human Kupffer cells (KC) co-cultured with primary human hepatocytes (PHHs) for PhysioMimix® DILI assay kit: human 24.

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 14 days by single-organ PhysioMimix® OOC System.

Protocol

Co-cultures of PHH donor (Lot # 121678) and KCs (Lot # 287345) were seeded into a PhysioMimix Multi-chip Liver-12+ plate on day 0 at 4×10^5 and 4×10^4 cells per well, respectively.

To assess cell health throughout the experiment, LDH, urea, albumin and CYP3A4, were measured at each media change (days 4, 6, 8, 11 and 14, see **Fig 1**).

The co-culture was treated with 100 ng/mL LPS or left untreated on days 7 and 13. IL-6 was measured on days 7, 8, 11 and 14 to assess the KCs inflammatory response (**Fig 2**).

On day 14 the scaffolds within each well of the Multi-chip Liver-12+ plate were removed, the microtissues within the scaffold's pores were fixed and imaged using a brightfield microscope (4x magnification) to assess the quality of the tissue formation (**Fig 3**). The qualification followed our internal document 001153_Rev01 KC Validation Protocol.

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Validation Data

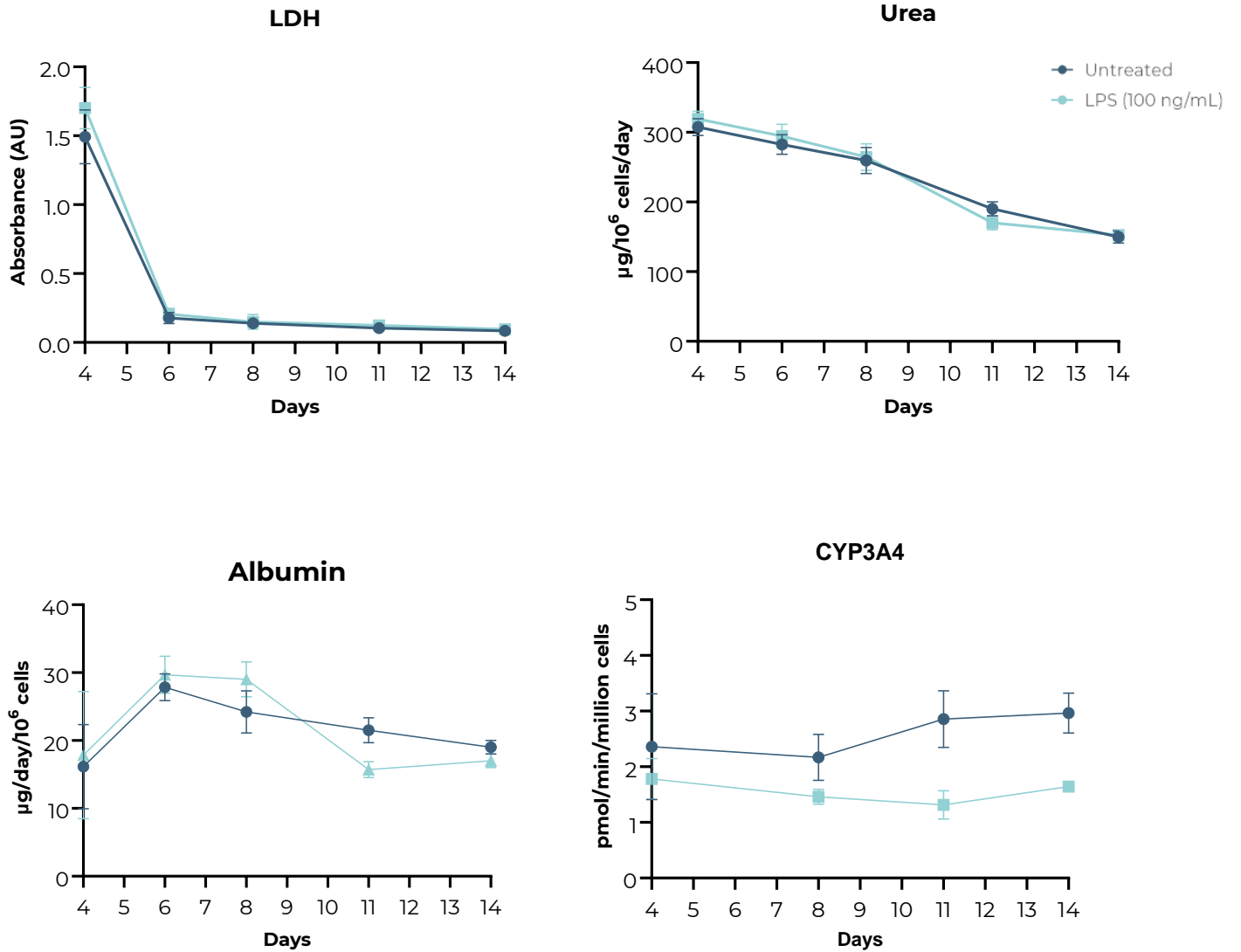


Figure 1 – Cell health and functionality analysis. Quantification of LDH, urea, albumin and CYP3A4 within media samples at days 4, 6, 8, 11 and 14 of the culture. Data presented as mean with SD from triplicate samples.

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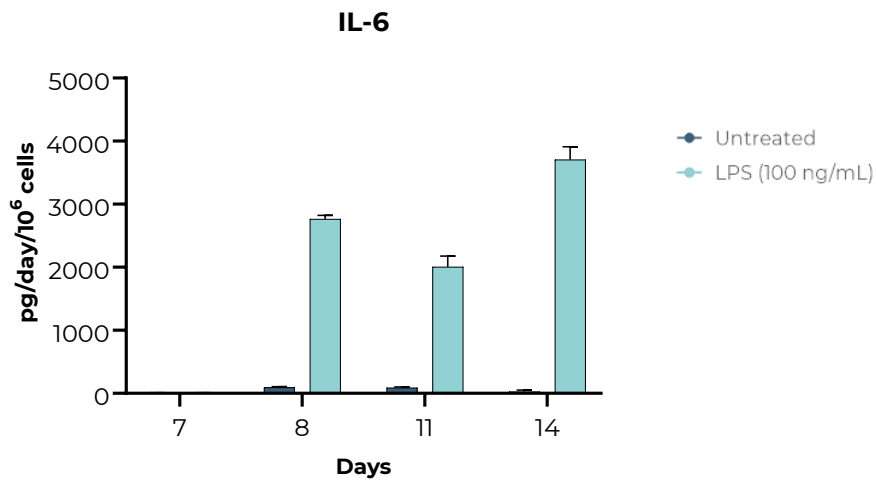


Figure 2 – Cell inflammatory response analysis. Quantification of IL-6 within media samples taken at days 7, 8, 11 and 14 of the culture, measured by ELISA. Data presented as mean with SD from triplicate samples.

Microtissue formation

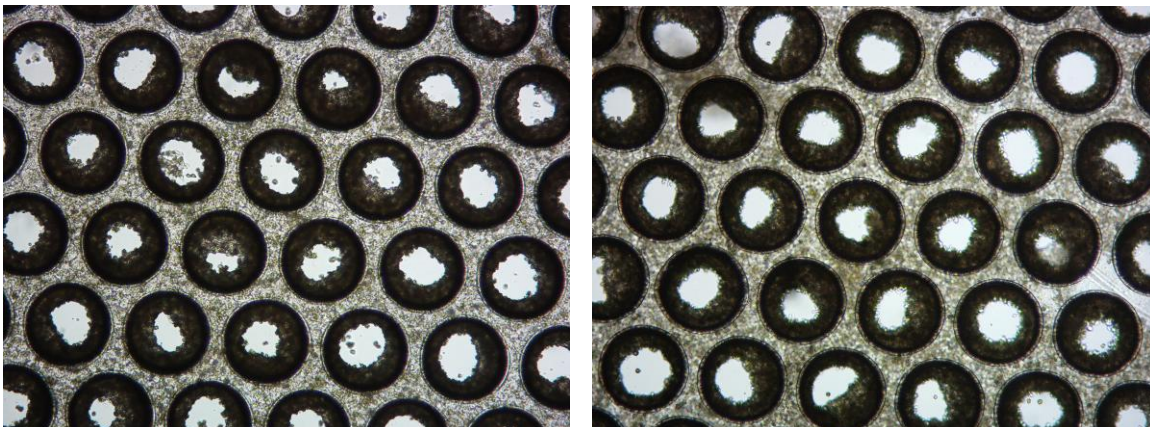


Figure 3 – Brightfield Microscopy. Representative image taken from one scaffold removed from the Liver-12+ plate at day 14.