emulate

Method for Liver-Chips:

Alanine Transaminase Protein Quantification Assay

EM143 rev. A

Goals:	Key Steps:	Other Required Materials:
Quantitative measurement of human alanine transaminase (ALT) protein from Emulate human Liver-Chip effluent samples	1. Prepare all reagents, samples, and standards 2. Run the assay 3. Read the plate	• Human ALT ELISA Kit Colorimetric / (Abcam Cat No. ab 234578) • Plate reader

1. Method

Sample type	Liver-Chip effluent See Protocol EP124 Emulate Effluent Sampling.
Liver-Chip assay flow rate (recommended)	The media should flow at a rate of 30 µL / h
Liver-Chip effluent dilution (recommended)	These samples should *not* be diluted – rather, they should be loaded neatly. Note: Sample dilution may require further adjustment to accommodate for any experimental modifications by the user or for cells from alternative donors.
Run assay as described on supplier site	Visit the following link for the supplier's instructions: https://www.abcam.com/human-alt-elisa-kit-ab234578.html
Liver-Chip data range	Liver-Chip data should fall within a range of 0-155 ng per day, per million cells (0-155 ng / day / million cells)
Converting pg/mL to ng/day/million cells	1. Multiply the determined pg / mL value by 1 million cells. Note: Before performing this calculation, multiply the initial value by the dilution factor, if one exists. 2. Divide by 42,000 (the cells in chip) 3. Multiply by the total mL collected 4. Divide by 1,000 If X equals the determined pg / mL value (after multiplying by dilution factor), and $V_{\text{total collected [in mL]}}$ = 0.72 mL in 24 hours with a flow rate of 30 µL / h, then ng / day / million cells = $X*(1{,}000{,}000/42{,}000)*(V_{\text{total collected [in mL]}}/1000)$

Considerations for effluent processing and data analysis	1) The ALT protein is sensitive to temperature fluctuations. Caution should be taken when collecting and storing effluent samples to ensure that most of the secreted protein is preserved for quantification. a. Aspirate the outlet reservoirs 24 hours prior to collecting the effluent for ALT assay to ensure that freshly secreted protein is used for analysis and that minimal degradation occurs at 37°C. b. Store samples at -80°C to preserve protein and limit degradation. c. Aliquot effluent samples in multiple plates to avoid freeze and thaw cycles, which can lead to substantial protein degradation and misleading results. 2) The ALT levels detected from the effluent will change depending on cell injury status, the number of viable cells remaining on chip, and donor-to-donor variability. a. The amount of ALT released in the media will be directly proportional to the number of live cells on the chip. Therefore, to accurately quantify ALT levels across different treatment groups, the user should perform data normalization by cell number on chip (counting DAPI signals) or cell mass (protein amount from cell lysate). b. In general, we expect ALT levels to increase on the chip due to hepatocyte toxicity. However, severe hepatocyte injury can also result in massive cell death, which in turn causes less ALT to be secreted on the effluent. In extremely toxic conditions, ALT levels of 0 indicate that few viable cells remain on the chip.