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Protocol for Emulate Organ-Chips:

Multiplex Cytokine Assay

March 28, 2019

EP141 v1.0

TITLE Multiplex Cytokine Assay	DOCUMENT EP141	VERSION 8.0
	DATE 28-MAR-2019	PAGE 2 OF 2

Goals:	Key Steps:	Other Required Materials:
Detect and measure secreted cytokines and chemokines from Emulate Organ-Chip effluent samples	<ul style="list-style-type: none"> Prepare all reagents, samples, and standards Run the assay Read the plate 	<ul style="list-style-type: none"> U-PLEX® Biomarker Group 1 Human Assays (MSD® Cat No. K15067L) MSD instrument (electrochemiluminescence detection technology)

1. Method

Sample type	Organ-Chip effluent See Protocol EP124 Effluent Sampling.
Recommended assay flow rate (Liver-Chip)	30 µL / h
U-PLEX assay design	Assay plates can be customized by the user based on endpoint of interest. Measured cytokines in the Liver-Chip typically include: TNF-alpha, IL-1beta, IL-6, IL-8, IP-10, and MCP-1.
Liver-Chip effluent dilution (recommended)	No dilution: samples are loaded neat. Note: the dilution may be different if using different cell types.
Run assay as described on supplier site	https://www.mesoscale.com/en/products/u-plex-biomarker-group-1-human-assays-k15067l/ Note: The U-PLEX Biomarker Group 1 (hu) contains 74 cytokines and chemokines. Up to 10 assays from this assay group may be multiplexed on each plate for simultaneous quantification.
Sample concentration range (Liver-Chip)	<ul style="list-style-type: none"> TNF-α ~ 2-4 pg / mL IP-10 ~ 1- 60 pg / mL MCP-1 ~ 2–1,500 pg / mL IL-6 ~ 0.5-130 pg / mL

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