



emulate

Protocol for Emulate Organ-Chips:

Quantification of Cytokines and Acute Inflammatory Phase  
Proteins Secretion

April 8, 2021

EP220 v1.0

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Goals:	Key Steps:	Other Required Materials:
Quantify secretion levels of cytokines <b>and</b> acute inflammatory phase proteins and in Emulate Colon Intestine-Chip effluent samples	<ul style="list-style-type: none"> <li>• Prepare all reagents, samples, and standards</li> <li>• Run the assay</li> <li>• Read the plate</li> </ul>	<ul style="list-style-type: none"> <li>• V-PLEX Vascular Injury Panel 2 Human Kit (Meso Scale Diagnostics, Cat # <a href="#">k15053d</a>)</li> <li>• V-PLEX Human Proinflammatory Panel II (4-Plex) (Meso Scale Diagnostics, Cat # <a href="#">k15053d</a>)</li> <li>• Meso Scale Discovery plate reader</li> <li>• Multi-channel P200 pipette, P20–P1000 pipettes and tips</li> </ul>

## Introduction

This protocol uses the Colon Intestine-Chip as a reference point aiming to the quantitation of the secretion levels of cytokines (IL-1 $\beta$ , IL-6, IL-8, TNF $\alpha$ ) and acute inflammatory phase proteins (SAA, CRP, VCAM-1, ICAM-1) under baseline conditions and upon basolateral stimulation with the pro-inflammatory cytokine interferon gamma (IFN $\gamma$ ). These methods and assay conditions could change with a different Organ-Chip.

## Method

Sample type	Organ-Chip effluent samples See Emulate Protocol <a href="#">EP124 Effluent Sampling</a>
Recommended assay flow rate (Colon Intestine-Chip)	30 $\mu$ L/h
Recommended protein sample dilution (Primary Colon Intestine-Chip)	V-PLEX Vascular Injury Panel 2 Human Kit: Perform a 5-fold dilution. V-PLEX Human Proinflammatory Panel II (4-Plex): Perform a 2-fold dilution. Dilute samples in the respective diluent as indicated in the documentation provided by the manufacturer. <b>Note:</b> Secretion levels of the cytokines and acute inflammatory phase proteins will change depending on cell injury status or based on donor-to-donor variability. Therefore, sample dilutions may need to be modified to accommodate different experimental conditions or cells from different donors.
Run assay as described on manufacture instructions	<a href="https://www.mesoscale.com/en/products/v-plex-human-proinflammatory-panel-ii-4-plex-k15053d/">https://www.mesoscale.com/en/products/v-plex-human-proinflammatory-panel-ii-4-plex-k15053d/</a> <a href="https://www.mesoscale.com/en/products/v-plex-vascular-injury-panel-2-human-kit-k15198d/">https://www.mesoscale.com/en/products/v-plex-vascular-injury-panel-2-human-kit-k15198d/</a> <b>Note:</b> Store kit at 4°C and -20°C immediately upon receipt, according to manufacturer instructions.
Range of stimulation	IFN $\gamma$ : 10-100 ng/mL

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