Protocol for Emulate Organ-Chips:

Assessment of Cleaved Caspase-3

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EP211 v1.0

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Goals:	Key Steps:	Other Required Materials:
Assess cleaved Caspase 3 levels in Emulate Primary Colon Intestine-Chip epithelial cells lysates	• Prepare all reagents, samples, and positive and negative control samples • Run the assay • Read the plate	• Cleaved/Total Caspase-3 Whole Cell Lysate Kit (Meso Scale Diagnostics, cat# K15140D) • MSD Protein Lysis Buffer (cat# R60TX-3) • Meso Scale Discovery plate reader • Multi-channel P200 pipette, P20–P1000 pipettes and tips

Introduction

This protocol uses the Colon Intestine-Chip as a reference point aiming to the semi-quantitative assessment of the cleaved Caspase 3 levels under baseline conditions and upon basolateral stimulation with pro-inflammatory cytokines. These methods and assay conditions could change with a different Organ-Chip. Please reach out to Emulate Field Science Support for additional guidance.

Method

Sample type	Organ-Chip epithelial cells protein lysate See Emulate Protocol EP210 Protein Sample Lysing.
Recommended assay flow rate (Colon Intestine-Chip)	30 µL/h
Recommended protein sample dilution (Colon Intestine-Chip)	Dilute samples in protein lysis buffer to a final concentration of 400 µg/mL. Note: Cleaved Caspase-3 levels 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 supplier site	https://www.mesoscale.com/en/products/cleaved-total-caspase-3-whole-cell-lysate-kit-k15140d/ Note: Store kit at 4°C immediately upon receipt.
Range of stimulation	IFNγ: 10-100 ng/mL (36h – 72h) IL-22: 1nM (24h – 72h)