



emulate

Protocol for Emulate Organ-Chips:

Assessment of Phosphorylated Signal transducer and
activator of transcription 3 (STAT3)

April 8, 2021

EP218 v1.0

TITLE Assessment of Phosphorylated Signal Transducer and Activator of Transcription 3 (STAT3)	DOCUMENT EP218	VERSION 1.0
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Goals:	Key Steps:	Other Required Materials:
Assess phosphorylated STAT3 levels in Emulate Colon Intestine-Chip epithelial cells lysates	<ul style="list-style-type: none"> • Prepare all reagents, samples, and positive and negative control samples • Run the assay • Read the plate 	<ul style="list-style-type: none"> • Phospho-STAT3 (Tyr705) Kit (Meso Scale Diagnostics, Cat. # K150SVD-1) • Total STAT3 Kit (Meso Scale Diagnostics, Cat. # K150SND-1) • Phospho-STAT3 (Tyr705) Whole Cell Lysate Set (Meso Scale Diagnostics, Cat. # C11DI-1) • Meso Scale Discovery plate reader • Multi-channel P200 pipette, P20–P1000 pipettes and tips

Introduction

This protocol uses the Primary Colon Intestine-Chip as a reference point aiming to the semi-quantitative assessment of the phosphorylated Signal transducer and activator of transcription 3 (STAT3) levels under baseline conditions and upon basolateral stimulation with the cytokine interleukin 22 (IL-22). These methods and assay conditions could change with a different Organ-Chip or stimulant.

Method

Sample type	Organ-Chip epithelial cells protein lysate See Emulate Protocol Protein Sample Isolation .
Recommended assay flow rate (Primary Colon Intestine-Chip)	30 μ L/h
Recommended protein sample dilution (Colon Intestine-Chip)	Dilute samples, positive and negative control samples in protein lysis buffer to a final concentration of 800 μ g/mL. Note: Phosphorylated STAT3 levels will change depending on the applied stimulant, the 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/total-stat3-kit-k150snd/ https://www.mesoscale.com/en/products/phospho-stat3-tyr705-kit-k150svd/ https://www.mesoscale.com/~media/files/product%20inserts/phospho%20stat3%20lysates.pdf Note: Store kit at 4°C immediately upon receipt.
Range of stimulation	IL-22: 0.1 - 1nM

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