emulate

Protocol for Emulate Organ-Chips:

Protein Sample Isolation

April 8, 2021

EP210 v1.0

Goals:	Key Steps:	Other Required Materials:
Lysis and isolation of the protein fraction from epithelial cells in Emulate Primary Intestine-Chip.	• Lyse epithelial cells in the chip • Purify the protein fraction from the lysates	• MSD Tris Lysis Buffer (Meso Scale Diagnostics, R60TX-3) • Halt™ Protease Inhibitor Cocktail, EDTA-free (100X) (ThermoFisher Scientific, Cat No: 78425) • Phosphatase Inhibitor Cocktail 2 (Millipore Sigma, Cat No: P5726) • Phosphatase Inhibitor Cocktail 3 (Millipore Sigma, Cat No: P0044) • TrypLE™ Express Enzyme (1X), no phenol red (ThermoFisher Scientific, Cat No: 12604013) • DPBS • 1.5mL Eppendorf tubes • Microcentrifuge • P20–P1000 pipettes and tips

Introduction

This is the method we have developed for lysing and purification of the protein fraction for the epithelial cells of our Colon Intestine-Chip. For lysing and purification of customer-originated cell material, this protocol may need to be optimized. Please reach out to Emulate Field Science Support for additional guidance. This protocol has been optimized for identification of intracellular proteins using the Meso Scale Discovery technology.

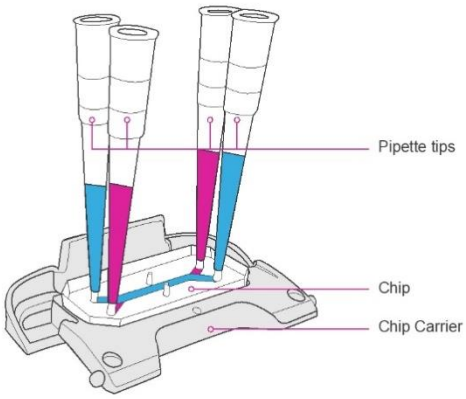
Method

Part I — Lysing of epithelial cells in the Colon Intestine-Chip

Reagent	Volume for One Chip (µL)
MSD Tris Lysis Buffer	194
Halt™ Protease Inhibitor Cocktail, EDTA-free (100X)	2
Phosphatase Inhibitor Cocktail 2	2
Phosphatase Inhibitor Cocktail 3	2
Total	200

1. Prepare the workspace of the chemical fume hood prior to beginning your work, ensuring that the space within the hood is organized, free from clutter, and the path of airflow is not blocked.
2. Ensure all chip carriers are labeled and identify the different conditions clearly. Detach chips from Pod™ modules and organize them in petri dishes for handling.
3. Pre-label all 1.5 mL tubes with its respective chip identification
4. Gently wash the apical channel once

   Note: For Cleaved Caspase-3, do not wash the channel. Instead, collect the apical super-natant of the top channel and place it in the pre-labeled 1.5 mL tube.
5. Place a 200 µL tip gently in the outlet of the bottom channel. We recommend using filtered tips for this step. Be careful not to push the tips too hard against the bottom of the chip channel as this could seal off the outlet and prevent reagents from going through the channel and outlet.



6. Add 50 µL of TrypLE in the bottom channel from the inlet, leaving the tips inserted into the inlet as shown in the diagram above.
7. Incubate for 2 minutes at room temperature.
8. Triturate, remove tips, and wash the bottom channel with 200 µL DPBS but leave the channel empty.
9. Place a 200 µL tip gently in the outlet of the top channel as described on step 4.
10. Add 75 µL of protein lysing solution, prepared as shown above, in the top channel from the inlet, leaving the tips inserted into the inlet as shown in the diagram above.
11. Incubate for 5 minutes on ice.
12. After incubation, triturate and use one of the two tips to collect the cell lysate in a clean 1.5 mL Eppendorf tube.
13. Using a new tip add 75 µL of protein lysing solution in the top channel from the inlet, leaving the tips inserted into the inlet as shown in the diagram above.
14. Incubate for 15 minutes on ice. Check under the microscope to confirm that the epithelial cells have been detached from the membrane. If not, extend the incubation time.
15. After incubation, triturate and use one of the two tips to collect the cell lysate in the same 1.5 mL Eppendorf tube.
16. Store the lysate in -80°C or proceed immediately to the purification of the protein fraction.

Part II — Purification of the Protein Fraction

1. Bring the protein lysate samples on ice.
2. Centrifuge at 16,000 x g for 20 minutes.
3. Transfer the supernatant into a fresh Eppendorf tube. That is the purified protein fraction.