emulate

Protocol for Emulate Organ-Chips:

Total Protein Quantification

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EP214 v1.0

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Goals:	Key Steps:	Other Required Materials:
Quantify the total amount of protein in the Emulate Colon Intestine-Chip.	• Prepare all samples and standards • Run the assay • Read the plate	• Pierce™ Coomassie Plus (Bradford) Assay Kit (Thermo Fisher Scientific, Cat. #: 23236) • DPBS • 96-well flat bottom plate • 1.5 mL Eppendorf tubes • Multi-channel P200 pipette, P20–P1000 pipettes and tips • Microtiter plate reader

Introduction

This is the method to identify the total amount of protein in epithelial cells lysate and/or effluent samples of the Colon Intestine-Chip. For protein quantification of customer originated cell material, this protocol may need to be optimized. Please reach out to Emulate Field Science Support for additional guidance.

Method

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. Thaw the protein samples on ice. See Protein Sample Isolation Protocol.
3. Prepare the albumin (BSA) standards, corresponding to a working range of 125 – 1500 µg/mL, as per the manufacturer's instructions.
4. Pipette 10 µL of the standards in the 96-well plate. Use technical duplicates.
5. Pipette the unknown protein samples in the 96-well plate by performing a 2-fold dilution with DPBS (5 µL sample + 5 µL DPBS). Use technical duplicates.

   Note: This dilution has been optimized for the identification of the total amount of protein in protein samples purified from lysates of epithelial cells cultured in the Colon Intestine-Chip (~200,000 - 300,000 cells/ chip). The dilution may need to be adjusted when assessing the total amount of protein of another cell type (e.g., endothelial cells) or in another Organ-Chip.
6. Add 300 µL of the Coomassie Plus Reagent to each well and incubate the plate in dark for 15 minutes at room temperature.
7. Measure the absorbance at 595 nm with a plate reader.
8. Prepare the standard curve and calculate the total protein concentration of the unknown samples according to the manufacturer's guidelines.