Protocol for Emulate Organ-Chips:

Cell Lysis for Protein Extraction

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EP135 v1.0

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Cell Lysis for Protein Extraction	EP135	1.0
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Goals:	Key Steps:	Other Required Materials:
Cell lysis in Organ-Chips for protein extraction	• Cell lysis	• Lysis buffer (recommended reagents: MSD Tris Lysis Buffer [#R60TX-3] or ThermoFisher RIPA buffer [#89900]) • PBS (ice cold) • Eppendorf Tubes® • Conical tubes

Notes:

- Conditions for protein extraction (e.g., choice of lysis and extraction buffer, incubation time, etc.) must be adjusted by the user according to the nature of the proteins of interest and the assays to be run on those samples. If you have a preferred or optimized lysis and extraction buffer and protocol, please use that in conjunction with general chip handling directions described below.
- SDS may be added to the lysis buffer to maximize the yield of soluble proteins. SDS extracts can be used for SDS electrophoresis and Western blotting. It is recommended to avoid the addition of SDS for 2D electrophoresis, enzyme-linked immunosorbent assay, and mass spectrometry.
- Protease and phosphatase inhibitors may be added to the lysis buffer to prevent or minimize protein degradation.
- In general, we recommend carrying out all steps for protein extraction at 2–8 °C.

1. Method

1. Remove Organ-Chips from Pod™ Portable Modules. Ensure all chip carriers are appropriately labeled before disconnecting chips from Pods.
2. Rinse both channels once with 200 µL of ice cold PBS.
3. Block inlet and outlet of the channel opposite the channel of interest with empty 200 µL tips. The channel of interest is the channel that contains cells from which proteins are being extracted. For example refer to Figure 1 below.
4. Review the specifications of the buffer(s) you will be using to determine the volume needed (usually 50–150 µL), the duration of lysis and extraction (usually 30 seconds to 10 minutes), and the recommended temperature (usually 4°C).
5. Gently wash the channel of interest once again with 200 µL of ice cold PBS. After washing, gently aspirate the PBS from the channel, leaving it dry. Then move on to complete steps 6–8, working as quickly and steadily as possible.
6. Block the outlet port of the channel of interest with an empty 200 µL tip. Ensure the tip is not pushed completely against the bottom of the channel to allow for smooth flow of lysis and extraction buffers in and out of the pipette tip. Note: At this stage, there should be a total of three tips inserted into the chip's ports.
7. Add the appropriate volume of lysis and extraction buffer into the inlet port of the channel of interest using a 200 µL tip. Pipette up and down several times to thoroughly lyse all cells.
8. Collect the lysate in an Eppendorf Tube® and place on ice.

9. Visually assess the chip under a bright-field microscope to ensure all cells in the channel of interest have been lysed successfully. If cells or tissue debris appear to be present, repeat steps 6–7 to successfully lyse all cells and collect everything from the channel of interest.
10. Take an aliquot for total protein quantification and further analysis if needed. Use protein extracts immediately after lysate collection, or store samples at -80°C for downstream analysis.

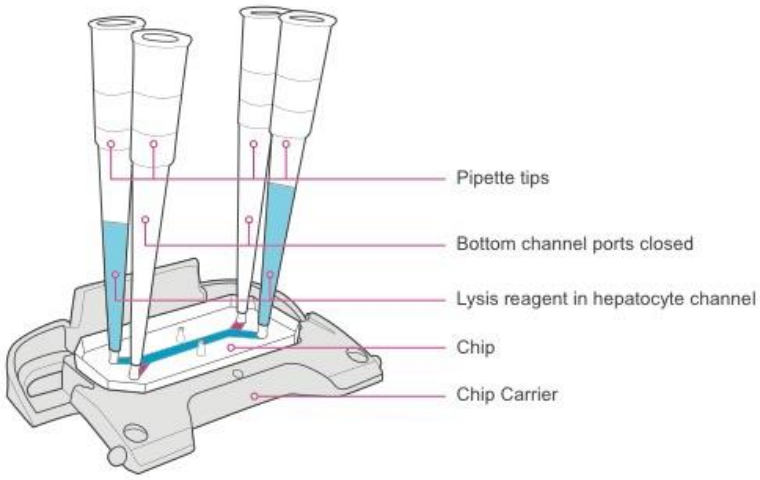

Figure 1:

- If the top channel is of interest, block the inlet and outlet of the bottom channel.
- If bottom channel is of interest, block the inlet and outlet of the top channel.