emulate

Protocol for Emulate Organ-Chips:

Cell Lysis for RNA Isolation

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EP161 v1.0

TITLE	DOCUMENT	VERSION
Cell Lysis for RNA Isolation	EP161	1.0
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Goals:	Key Steps:	Other Required Materials:
Lyse cells in Emulate Organ-Chips for RNA isolation	• Cell lysis	• Lysis buffer (recommended reagent: Thermo #12183018A) • Newly opened or designated sterile and RNAse-free PBS for RNA experiment (ice cold) • RNase-free Eppendorf Tubes® and tips

Introduction

Please follow the recommendations below to minimize RNA degradation and to maximize RNA yield.

- Work in a dedicated RNA isolation area.
- Wear disposable gloves while handling samples and reagents to prevent RNase contamination.
- Work quickly during sample harvesting and always use RNase-free tools and containers (tips, tubes, etc.).
- Use RNase decontamination solution to remove RNase contamination from work surfaces.

Method

1. Remove 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 cells are being lysed. (For example, refer to Figure 1 below)
4. Review the specifications of the lysis buffer you will be using to determine the volume needed (usually 50–150 µL), the duration of lysing (usually 30 seconds), and the recommended temperature (usually 4°C or room temperature).
5. Gently wash the channel of interest again with 200 µL of ice cold PBS. After washing, gently aspirate the PBS from the channel, leaving it dry. Then complete step 6 through step 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 buffer in and out of the pipette tip. Note: At this stage there should be a total of three tips inserted into ports of the chip.
7. Add the appropriate volume of lysis buffer using a 200 µL tip into the channel of interest (through the inlet port) and pipette up and down several times to thoroughly lyse all cells.
8. Collect the cell lysate in an RNase-free Eppendorf Tube® and place on ice.
9. Visually assess the chip under a brightfield microscope to ensure that all cells in the channel of interest have been lysed successfully. If cells or tissue debris appear to be present, repeat steps 6 and 7 to successfully lyse cells and collect lysate from the channel of interest.
10. Cell lysates can be used immediately for downstream RNA extraction or stored at -80°C.

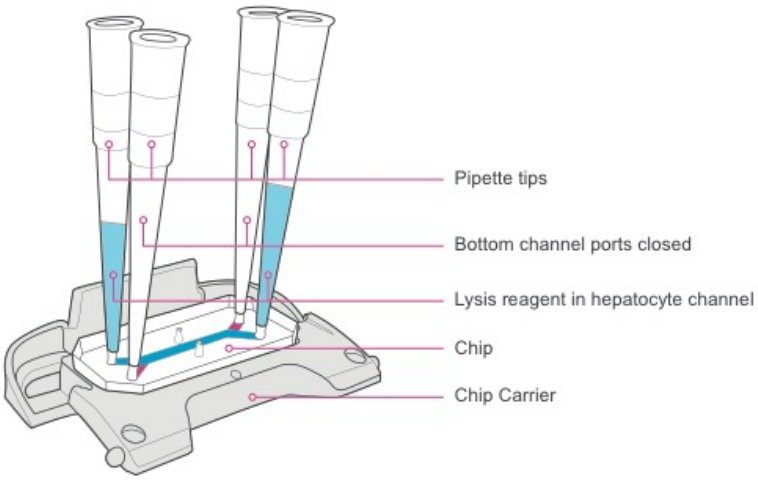

Figure 1

- 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.