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Organoid RNA Extraction

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We use this protocol and it's working

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Abstract

The following protocol is for collecting RNA from organoids in matrigel domes from a 12-well plate.

Lysing Organoids From a Plate

1 Remove the medium from the 3-5 fully confluent wells and use a p1000 to add fresh DMEM-F12 forecefully. Use the p1000 and extra DMEM added to break up the domes (note, for intestinal organoids, ice cold PBS can also be used). Triturate organoids with a 1 mL pipettor to break up the Matrigel® domes and fragment the organoids. For intestinal organoids, this will be about 20 titrations. Transfer the cells to the 15mL tube after going around the well multiple times with additional media.

2 Centrifuge at 300 x *g* for 5 minutes at room temperature (15 - 25°C).

3 Aspirate the supernatant, taking care to not disturb the pellet. If there is a translucent pellet above the organoid pellet, this is evidence that the Matrigel® might not be fully depolymerized. If so, aspirate the supernatant, add a fresh cold solution (e.g DMEM / F-12), and break up the pellet using a P1000 pipette. Add an additional 2 mL of cold solution and centrifuge at 300 x *g* for 5 min at 4°C. Note: Protein lysis buffer can be added to the pellet for subsequent western blot analysis.

4 Once the Matrigel® has been eliminated, aspirate the supernatant. Resuspend each pooled condition in 350 µL of Buffer RLT (from RNeasy Mini Kit)+ β-mercaptoethanol (β-ME) to lyse the organoid pellet (10uL of β-ME per 1mL of Buffer RLT).

5 Triturate the cell lysate until all visible organoid fragments are dissolved and the mixture first acquires a viscous consistency, then becomes slightly less viscous. Transfer the entire lysate volume to a QIAshredder spin column placed in a 2 mL collection tube.

6 Centrifuge the spin column at 10,000 x *g* for 2 minutes at room temperature. The spin column can be discarded. Cap the collection tube containing the homogenized lysate.

7 Transfer lysate into labeled collection tubes on ice. Note: The lysate can be stored at -80°C for up to 6 months prior to RNA isolation. Immediately store the sample at -80°C if not using immediately.

RNA Extraction

8 Add 1 volume of 70% ethanol to the homogenized lysate, and mix well by pipetting. **Important**: Do not centrifuge.

9 Transfer up to 700 µl of the sample, including any precipitate that may have formed, to an RNeasy spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through but not the

collection tube. If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same RNeasy spin column. Discard the flow-through after each centrifugation.

10 Add 700 µl Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through but not the collection tube.

11 Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through. Reuse the collection tube.

12 Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane.

13 Place the RNeasy spin column in a new 2 ml collection tube (supplied), and discard the old collection tube with the flow-through. Close the lid gently, and centrifuge at full speed for 1 min.

14 Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 30–50 µl RNAse-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute the RNA.