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Fresh Cell Isolation RNA Extraction

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We use this protocol and it's working

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Abstract

This protocol describes the process of extracting RNA from freshly isolated immune cell populations using the Qiagen RNeasy Mini Kit.

Guidelines

Always wear gloves and spray with RNaseZap and spread solution all over gloves.
Make sure to use RNA only pipettes and filter tips.
Perform all steps of the procedure at room temperature. During the procedure, work quickly.
Perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

Materials

RNeasy mini kit (74106, Qiagen)
QIAshredder (79656, Qiagen)
96-100% Ethanol
Nuclease Free Water (AM9938,Thermo Scientific)
2-mercaptoethanol (M6250, Sigma Aldrich)
RNaseZAP(AM9780, Ambion)
1.5ml tubes

Safety warnings

Wear appropriate PPE and work with concentrated β-ME and ethanol in a chemical safety hood.

Before start

Prepare BME lysis buffer
Add 20 µl β-ME per 1 ml Buffer RLT. Dispense in a fume hood. Buffer RLT containing β-ME can be stored at room temperature for up to 1 month.

Prepare RPE buffer
Add 4 volumes of ethanol (96–100%) as indicated on the bottle to concentrate buffer RPE to obtain a working Solution.

RNA Extraction

1 Resuspend cell pellet in 350uL of BME+RLT lysis buffer and vortex well.

2 Transfer lysis solution to (purple) qiashredder tubes and then spin 21300xg (full speed) for 2min.

2.1 Can freeze flow-through of cell lysate at this stage and store in -80 degrees celsius for several months

3 Add 350uL of 70% ethanol to the homogenized lysate, and mix well by pipetting.

4 Transfer 700 µl of the sample, including any precipitate that may have formed, to an RNeasy spin column placed in a 2 ml collection tube. Close the lid gently, and centrifuge for 30s at 9,391xg (10,000rpm). Discard the flow-through .

5 Add 700 µL Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 30s at 9,391xg (10,000 rpm) to wash the spin column membrane. Discard the flow-through.

6 Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 30s at 9,391xg (10,000rpm) to wash the spin column membrane. Discard the flow-through.

7 Repeat step 6.

8 Spin for 2 minutes at full speed to ensure any residual buffers are gone.

9 Place the RNeasy spin column in a new 1.5 ml collection tube. Add 20µl RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at 9,391xg (10,000rpm) to elute
the RNA.

10 After first spin transfer elution back to the membrane of the RNeasy spin column and spin again for 1 min at 9,391xg (10,000rpm) (re-elution results in greater RNA yield). Samples are now ready for downstream assays or should be stored at -80 degrees.