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 We use this protocol and it's working

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Qiagen - RNeasy mini kit for cells

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MATERIALS

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⊗ Buffer RPE **Contributed by users**

⊗ RLT Buffer **Qiagen**

⊗ Ethyl Alcohol **Sigma Catalog #E7023**

⊗ RNase-free water **Contributed by users**

⊗ RW1 buffer **Qiagen Catalog #74106**

⊗ Ethyl alcohol 70% **Contributed by users**

BEFORE START INSTRUCTIONS

Clean the benches and all the material that will be used with alcohol 70.

Use tips with filter.

Add 4 volumes of 100% ethanol to the RPE buffer.

To lyse the cells, you can use β -mercaptoethanol or 2 M dithiothreitol. For each 1ml of the RLT buffer, add 10 μ l of β -mercaptoethanol. For each 1ml of the RLT buffer, add 20 μ l of 2 M dithiothreitol.

You must use a maximum of 1×10^7 cells.

RNA extraction

- 1 If you are using less than 5×10^6 add 350 μl of the RLT buffer prepared above. If the number of cells is greater than this, use 700 μl .
- 2 Homogenize the samples using vortex or use QIAshredder. If necessary use an insulin syringe to break the cells.
- 3 Add 1 volume of 70% ethanol to the lysate and homogenize with the pipette.
- 4 Transfer up to 700 μl of the sample, including any precipitate, to an RNeasy Mini spin column placed in a 2 ml collection tube.
- 5 Centrifuge for 15 seconds at 8000 g. Discard the flow-through.
- 6 Add 700 μl of the RW1 buffer to the column and centrifuge for 15 seconds at 8000 g. Then discard the flow-through.
- 7 Add 500 μl of the RPE buffer to the column and centrifuge for 15 seconds at 8000 g. Then discard the flow-through.
- 8 Add 500 μl of the RPE buffer to the column and centrifuge for 2 minutes at 8000 g.
- 9 This step is optional. Place the column in a new 2 mL collection tube and centrifuge at full speed

for one minute to dry the membrane.

- 10** Place the column in a new 1.5 mL collector tube and add 30 to 50 µl RNase Free water and centrifuge for 1 minute to 8000 g to elute the RNA.
- 11** If you expect to have more than 30 µg of RNA, repeat step 10 again using 30 to 50 µl of RNase-free water. Or, use the elution you acquired in step 10. Reuse the pickup tube from step 10.
- 12** Stock the sample at -80 ° C.