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Qiagen - RNeasy mini kit for cells

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Maysa Silva[1], Maryana Branquinho[1], Maria Cármen Sales[1]

[1]Universidade de São Paulo

Maysa Silva

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Protocol status: Working
We use this protocol and it's working

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Materials

MATERIALS

- Buffer RPE
- RLT Buffer **Qiagen**
- Ethyl Alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E7023
- RNase-free water
- RW1 buffer **Qiagen Catalog #**74106
- Ethyl alcohol 70%

Before start

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 µl of β-mercaptoethanol. For each 1ml of the RLT buffer, add 20 µl 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 RNAsse 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 step10 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.