# Qiagen - RNeasy mini kit for cells

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## Abstract

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## 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 x $10^7$ cells.

## Materials

- Buffer RPE by Contributed by users
- RLT Buffer by Qiagen
- Ethyl Alcohol E7023 by Sigma
- RNase-free water by Contributed by users
- RW1 buffer 74106 by Qiagen
- Ethyl alcohol 70% by Contributed by users

## Protocol

### RNA extraction

***Step 1.***

If you are using less than 5 x $10^6$ add 350 µl of the RLT buffer prepared above. If the number of cells is greater than this, use 700 µl.

### RNA extraction

***Step 2.***

Homogenize the samples using vortex or use QIAshredder. If necessary use an insulin syringe to break the cells.

RNA extraction

***Step 3.***

Add 1 volume of 70% ethanol to the lysate and homogenize with the pipette.

RNA extraction

***Step 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.

RNA extraction

***Step 5.***

Centrifuge for 15 seconds at 8000 g. Discard the flow-through.

RNA extraction

***Step 6.***

Add 700 µl of the RW1 buffer to the column and centrifuge for 15 seconds at 8000 g. Then discard the flow-through.

RNA extraction

***Step 7.***

Add 500 µl of the RPE buffer to the column and centrifuge for 15 seconds at 8000 g. Then discard the flow-through.

RNA extraction

***Step 8.***

Add 500 µl of the RPE buffer to the column and centrifuge for 2 minutes at 8000 g.

RNA extraction

***Step 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.

RNA extraction

***Step 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.

## RNA extraction

***Step 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.

## RNA extraction

***Step 12.***

Stock the sample at -80 ° C.