Qiagen- RNeasy Mini Kit for tissue

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Abstract

Protocol for extraction of tissue RNA by Qiagen Mini Kit.

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Before start

Clean the benches and all the material that you will use with alcohol 70.
Use tips with filter.

Add 4 volumes of ethanol 100 to 4 volume RPE buffer.

To lyse your sample, work with β-mercaptoethanol or 2 M dithiothreitol.
For β-mercaptoethanol, use 10 µl for every 1 ml of the RLT buffer.
For 2 M dithiothreitol, use 20 µl for every 1 ml of the RLT buffer.

Materials

- Buffer RPE by Contributed by users
- Ethanol 100% by Contributed by users
- RLT Buffer by Qiagen
- Ethanol 70% by Contributed by users
- RNase-free water by Contributed by users
- RW1 buffer 74106 by Qiagen

Protocol

RNA extraction

Step 1.

Do not use more than 30 mg of tissue. If you are using less than 20 mg add 350 µl of the RLT buffer prepared initially. If the mass is larger than this, use 700 µl.

RNA extraction

Step 2.

For disruption and homogenization use TissueLyser LT; TissueLyser II; TissueRuptor, or mortar and pestle followed by QIAshredder or needle and syringe.

RNA extraction

Step 3.

Add 1 volume of ethanol 70 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 collector tube of 1.5 mL and add 30 to 50 µl RNAsse Free water. 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 the previous step again using 30 to 50 µl of RNase-free water. Or, use the elution you acquired in the previous step. Reuse the manifold.

RNA extraction

Step 12.

Stock the sample at -80 ° C.