Aug 21, 2018

Qiagen- RNeasy Mini Kit for tissue

DOI
dx.doi.org/10.17504/protocols.io.ssceeaw

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DOI: https://dx.doi.org/10.17504/protocols.io.ssceeaw

Protocol Citation: Maysa Silva, Maryana Branquinho, Maria Cármen Sales 2018. Qiagen- RNeasy Mini Kit for tissue. **protocols.io https://dx.doi.org/10.17504/protocols.io.ssceeaw**

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

Created: August 21, 2018

Last Modified: August 21, 2018

Protocol Integer ID: 14884

Keywords: rna extraction, mini kit qiagen, tissue, qiagen mini kit, extraction of tissue rna, tissue rna, rneasy mini kit, qiagen, rna, tissue protocol

Abstract

Protocol for extraction of tissue RNA by Qiagen Mini Kit.

Materials

MATERIALS

- Buffer RPE
- Ethanol 100%
- RLT Buffer **Qiagen**
- Ethanol 70%
- RNase-free water
- RW1 buffer **Qiagen Catalog #**74106

Troubleshooting

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.

RNA extraction

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.

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

3 Add 1 volume of ethanol 70 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 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.

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.

12 Stock the sample at -80 ° C.