AUG 21, 2018

🌐 Qiagen- RNeasy Mini Kit for tissue

Maysa Silva¹, Maryana Branquinho¹, Maria Cármén Sales¹

¹Universidade de São Paulo

Maryana Branquinho

ABSTRACT

Protocol for extraction of tissue RNA by Qiagen Mini Kit.

MATERIALS

MATERIALS

⊗ Buffer RPE **Contributed by users**

⊗ Ethanol 100% **Contributed by users**

⊗ RLT Buffer **Qiagen**

⊗ Ethanol 70% **Contributed by users**

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

⊗ RW1 buffer **Qiagen Catalog #74106**

BEFORE START INSTRUCTIONS

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 μ l for every 1 ml of the RLT buffer.

For 2 M dithiothreitol, use 20 μ l for every 1 ml of the RLT buffer.

OPEN ACCESS

DOI:

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

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

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Aug 21, 2018

Last Modified: Aug 21, 2018

PROTOCOL integer ID:
14884

Keywords: rna extraction,
mini kit qiagen, tissue

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 RNase 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.