Dec 28, 2020

RNA Extraction

Alicia M Rich[1]

[1]Otterbein University

In Development This protocol is published without a DOI.

PAL Lab Otterbein
Tech. support email: **rich2@otterbein.edu**


Alicia Rich
Otterbein University

PROTOCOL CITATION

Alicia M Rich 2020. RNA Extraction. **protocols.io**
https://protocols.io/view/rna-extraction-bq3emyje

CREATED

Dec 28, 2020

LAST MODIFIED

Dec 28, 2020

PROTOCOL INTEGER ID

45894

GUIDELINES

Complete all steps in the biosafety cabinet.

MATERIALS TEXT

RNEasy Plus Mini

Kit **Qiagen Catalog #74134**

- gDNA Eliminator Mini Spin Columns (uncolored)
- RNeasy Mini Spin Columns (pink)
- Collection Tubes (1.5 ml)
- Collection Tubes (2 ml)
- Buffer RLT Plus
- Buffer RPE (concentrate - add ethanol)
- RNase-Free Water

RLT Plus contains a guanidine salt. Do not get any of the bleach close to it or let them mix.

Supplies not in the kit:

- QIAshredder **Qiagen** Step 5
- Sterile, RNase-free pipet tips
- Microcentrifuge (only use the biohazard one in the extraction area of the lab)
- 96-100% ethanol
- 50% ethanol in sterile water
- Nitrile gloves

SAFETY WARNINGS

When working with primate tissue, always wear a facial covering, nitrile examination gloves, and lab coat. Do not open tissue samples outside the biosafety cabinet and make sure you are properly trained in maximizing the cabinet's utility first.

DISCLAIMER:

BEFORE STARTING

Wipe down your workspace with a10% bleach solution or RNAse spray.

After setting up all of your reagents and tools in the cabinet, close the hood and run the UV light for 15 minutes.

Add 4 volumes of RNA-safe 100% ethanol to 4 volume ⊠Buffer RPE **Qiagen Catalog #1018013** .

Add **10 µl** ⊠β-Mercaptoethanol **Contributed by users Catalog #M3148** to the ⊠RLT Buffer **Qiagen** .

Lyse and Homogenize 12m

1 Do not allow samples to thaw before adding the ⊠Buffer RLT Plus **Qiagen Catalog #1053393** . If a sample needs to be thawed for measuring, do so on ice.

2 Use sterilized forceps to transfer swab or ~90 mg of feces into a 2 ml microcentrifuge tube held on ice or in a cooling block.

When processing samples containing average or low amounts of RNA, the maximum amount of starting material shown in Table 1 (kit manual) can be used. However, even though the RNA binding capacity of the RNeasy spin column is not reached, the maximum amount of starting material must not be exceeded. Otherwise, lysis will be incomplete and cellular debris may interfere with the binding of RNA to the RNeasy spin column membrane, resulting in lower RNA yield and purity.

3 For feces: add **1.8 mL** ⊠Buffer RLT Plus **Qiagen Catalog #1053393** (or as much as you can filling the tube).

For swab: add **600 µl** ⊠Buffer RLT Plus **Qiagen Catalog #1053393** .

4

Place the tubes in the shaking block at maximum speed and minimum temperature for 20 minutes.

10000 rpm, 4°C, 00:20:00

5 For feces: transfer **700 µl** of the lysate into a QIAshredder **Qiagen** tube. Keep the rest of the lysate in the original tube on ice.

For swab: transfer the entire lysate volume and swab tip into a QIAshredder **Qiagen** tube.

6

Spin the column at the maximum speed for 2 minutes in the centrifuge at the extraction station.

10000 rpm, 00:02:00

7 For feces only: Transfer the lysate into another 2 ml microcentrifuge tube, and replace the same one with the same spin column.

8 For feces only:

go to step #5 Repeat until you have used all of the lysate or filled the 2 ml tube.

9

For all samples: Centrifuge the total lysate volume for 3 min at 10,000 rpm. **10000 rpm, 00:03:00**

Remove genomic DNA

10 Carefully remove the supernatant by pipetting, and transfer it to a gDNA Eliminator spin column placed in a 2 ml collection tube (included in the kit).

11

10000 rpm, 00:00:30 Centrifuge for 30 s at 10,000 rpm. Discard the column and save the flow-through.

11.1 Make sure that no liquid remains on the column membrane after centrifugation. If necessary, repeat this step until all liquid has passed through. **go to step #11**

12

Add **600 µl (or equal volume)** 50% ethanol to the flow-through, and mix well by pipetting. DO NOT CENTRIFUGE. Work quickly and proceed immediately to next step.

If some lysate was lost during the homogenization and DNA removal, adjust the volume of ethanol accordingly.

Precipitates may be visible after the addition of ethanol, but this will not affect the procedure.

Bind the RNA

13 Transfer up to **700 µl** of the sample, including any precipitate that may have formed, to an RNeasy spin column placed in a 2 ml collection tube (included in the kit). Keep any remaining sample in the same tube on ice.

14 

10000 rpm, 00:00:15 Centrifuge for 15 s at 10,000 rpm or faster. Discard the flow-through, but save the tube for the next step.

14.1 If any sample remained in the tube, repeat until all liquid has passed through the column.
go to step #13

Wash

15 Buffer

Add **700 µl** RW1 **Qiagen Catalog #1053394** to the RNeasy spin column with the same empty collection tube from the previous step. Close the lid gently.

16 

10000 rpm, 00:00:15 Centrifuge for 15 s at 10,000 rpm or faster to wash the column membrane. Discard the flow-through.

After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Be sure to empty the collection tube completely.

17 Buffer

Add **500 µl** RPE **Qiagen Catalog #1018013** to the RNeasy spin column with a fresh collection tube. Close the lid gently.

Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use.

18 

10000 rpm, 00:00:15 Centrifuge for 15 s at 10,000 rpm for faster. Discard the flow-through, but save the collection tube again for the next step.

19 Buffer

Add **500 µl** RPE **Qiagen Catalog #1018013** to the RNeasy spin column with the same emptied collection tube from the previous step. Close the lid gently.

20

Centrifuge for 2 min at 10,000 rpm or faster. **10000 rpm, 00:02:00**

Carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Otherwise, a carryover of ethanol will occur.

21

Place the RNeasy spin column in a new 2 ml collection tube, and discard the old collection tube with the flow-through. Centrifuge at full speed for 1 min. **10000 rpm, 00:01:00**

Elute 10m

10m

22

Place the RNeasy spin column in a new 1.5 ml collection tube, clearly labeled with the sample ID, date, and your initials. Add **70 µl** RNase-free water **Contributed by users** directly to the spin column membrane. Close the lid gently, and allow the tube to sit on ice for 10 minutes. **00:10:00**

23

10000 rpm, 00:01:00 Centrifuge for 1 min at 10,000 rpm to elute the RNA. DO NOT DISCARD THE FLOW-THROUGH OR COLUMN.

24 **go to step #22 with a second labeled collection tube and the same column.**

25 Immediately transfer both tubes to the freezer. **-80 °C** (**-20 °C** if you plan to carry out PCR immediately)