High-quality RNA purification with on-column DNase treatment from tissue specimens

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Jan 24, 2020

1 Works for me

dx.doi.org/10.17504/protocols.io.8ufhwtn

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ABSTRACT

This protocol can be used for total RNA purification from tissue samples. It includes an intermediate on-column DNase treatment, which results in higher RNA yield and purity. This method is mainly optimised to obtain hepacivirus RNA extracts for whole-genome sequencing, but it can also be used for any viral RNA purification from samples with lower viral loads.

GUIDELINES

- During tissue handling, all procedures should be carried out as quickly as possible.
- Only RNA molecules > 200 nucleotides are purified.
- Do not overload the RNeasy spin column (maximum capacity of **700 µl**).
- Buffer RLT may form a precipitate upon storage. Re-dissolve by putting the bottle under warm water for a few minutes.
- Perform all steps at **Room temperature** , unless otherwise stated.
- Always use new collection tubes to eliminate any possible contamination.

MATERIALS

NAME	CATALOG #	VENDOR
RNeasy Mini Kit	74104	Qiagen
RNase-Free DNase Set	79254	Qiagen
Precellys CK28 Lysing Kit Hard Tissue Homogenizing Bertin	10144-516	VWR International

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Precellys CK28 Lysing Kit Hard Tissue Homogenizing Bertin	10144-516	VWR International

BEFORE STARTING

- When using Buffer RPE for the first time, add 4 volumes of ethanol (100%).
- Prepare DNase I stock solution of RNase-free DNase set as described:

  Do not open the glass vial. Transfer **550 µl** of the RNase-free water provided into a **1.5 ml** Eppendorf tube. Using a needle and a syringe inject the water into the lyophilised DNase I glass vial. Mix gently by inverting the vial and make sure the powder on the sides of the vial is all well dissolved. **DO NOT VORTEX**.

  For long-term storage of DNase I, remove the stock solution from the glass vial, using the syringe. Divide it into **100 µl** aliquots and store at -- **20 °C** °C for up to 9 months. Thawed aliquots can be stored at **4 °C** for up to 6 weeks. Do not refreeze the aliquots after thawing.

Sample homogenisation

1 Add **600 µl** RLT Buffer to the Precellys lysate tubes.

Precellys CK28 Lysing Kit Hard Tissue Homogenizing Bertin
by VWR International
Catalog #: 10144-516

2 Excise a lentil-sized piece of tissue (maximum amount of **20 mg** for RNAlater stabilized tissues and **30 mg** for fresh or frozen tissues) and transfer it quickly to the lysate tubes. Make sure that all tissues are immersed into the RLT reagent.

3 Place tubes on dry ice for **00:02:00** (or until frozen) and then thaw quickly.

4 Immediately after thawing disrupt the tissues using a conventional rotor-stator homogeniser (Minilys). The recommended lysis should be performed at medium speed (**4000 rpm**) for **00:02:00** .

Minilys Personal Homogeniser
Tissue homogeniser
Bertin Instruments **P000673-MLYS0-A**

5 Place tubes on dry ice for **00:02:00** (or until frozen) and then thaw quickly.
Check the results and repeat the homogenisation until no more visible fragments are present.

6 Centrifuge the lysate for **00:03:00** at full speed. Carefully remove the supernatant by pipetting, and transfer it to a new **1.5 ml** Eppendorf tube.

RNA binding

7 Add **600 µl** 70% ethanol to the supernatant, and mix immediately by pipetting 5 times. **DO NOT VORTEX OR CENTRIFUGE**. Proceed immediately to the next step.

8 Transfer **700 µl** of the sample to an RNeasy spin column placed in a **2 ml** collection tube. Centrifuge for **00:00:30** at **10000 rpm**

If the sample is more than **700 µl** , change collection tubes and transfer the rest to the spin column and centrifuge again. Discard the collection tubes and replace with new ones.

9 Add **350 µl** Buffer RW1 to the RNeasy spin column and centrifuge for **00:00:30** at **10000 rpm** to wash the spin column membrane. Change collection tubes.

DNase treatment

10 Prepare the DNase treatment master mix, as described:
For each sample, add **10 µl** DNase I stock solution to **70 µl** Buffer RDD. Mix by gently inverting the tube, and spin down briefly. **DO NOT VORTEX**.

11 Add the **80 µl** DNase I incubation mix directly to the RNeasy spin column membrane, and place on benchtop at **Room temperature** for **00:15:00** .

Washing steps

12 Add **350 µl** Buffer RW1 to the RNeasy spin column and centrifuge for **00:00:30** at **10000 rpm** to wash the spin column membrane. Change collection tubes.

13 Add **500 µl** Buffer RPE to the RNeasy spin column and centrifuge for **00:00:30** at **10000 rpm** . Change collection tubes.

14 Add **500 µl** Buffer RPE to the RNeasy spin column and centrifuge for **00:02:00** at **10000 rpm** . Change collection tubes.

Dry centrifugation

15 Place the RNeasy spin column in a new collection tube and centrifuge at full speed for **00:05:00** If there is still much liquid passing through the column, change collection tubes and centrifuge for **00:01:00** .

Elution and storage

16 Place the spin column in a new **1.5 ml** Eppendorf tube. Add **50 µl** RNase-free water directly to the spin column membrane. Incubate for **00:05:00** at **Room temperature** . Then centrifuge for **00:01:00** at **10000 rpm** to elute the RNA.

17 Repeat step 16. Use a new **1.5 ml** tube for another elution round of **50 µl** .

18 Store at -- **20 °C** . Optionally, you can put the column back to the corresponding collection tube and store them at -- **20 °C** for another future elution. Even after the 3rd or 4th elution time, there are still some RNA molecules passing through the column.