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RNA extraction using the PureLink® RNA Mini Kit

In 1 collection

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ASAP Collaborative Rese… | Enrico Protocols

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Abstract

This Protocol details the extraction of total RNA using the PureLink® RNA Mini Kit.

Materials

Materials:

- Purelink RNA mini kit
- 70% EtOH in molecular biology grade water
- Molecular biology grade water

Preparations:

1. Wash buffer 2: add 60 ml of 100% EtOH directly to the bottle.
2. Lysis buffer: prepare a fresh aliquot containing 1% 2-mercaptoethanol (or 40 mM DTT).

Prepare lysates from ≤5 × 10^6 monolayer cells:

7m 45s

1 Remove the growth medium from the cells.

Note

We used a 15 cm^2 dish for each sample. The cells, collected in 1 ml of DPBS, were then divided, with ⅔ used for western blotting, and ⅓ used for the following protocol for RNA extraction.

2 Using RNase-free pipette tips, add the appropriate volume of lysis buffer prepared with 2-mercaptoethanol to sample.

Before beginning the lysis and homogenization steps, prepare a fresh amount of Lysis Buffer containing 1% 2-mercaptoethanol for each purification procedure. Add 10 µL 2–mercaptoethanol for each 1 mL Lysis Buffer.

Using the table below, determine the correct amount of Lysis Buffer needed for your sample type and amount.

Note: For larger than average samples, or if using a rotor-stator, additional Lysis Buffer may be required. See page 12 for details.

Number of cells in your sample	Amount of Lysis Buffer Needed (prepared with 2-mercaptoethanol)
≤1 × 10^6	0.3 mL*
1 × 10^6–5 × 10^6	0.6 mL
5 × 10^6–5 × 10^7	0.6 mL per 5 × 10^6 cells For example: use 1.2 mL for 1 × 10^7 cells and 6.0 mL for 5 × 10^7 cells

*Use 0.6 mL if using rotor-stator for lysis or homogenization.

3 Proceed with one of the following homogenization options at Room temperature :

3.1 Transfer the lysate to a homogenizer inserted in a collection tube and centrifuge at 12000 x g, 00:02:00 . Remove the homogenizer when done, or

2m

3.2 **Transfer the lysate to a 1.5 mL RNase–free tube and pass 5–10 times through an 18-21 gauge needle attached to an RNase-free syringe**
Centrifuge the homogenate at ~ 2600 x g, 00:05:00 , then transfer the supernatant to a clean RNase-free tube

10m

Note

We homogenized the cells by 10 passages in a 20G needle in 300 µL of lysis buffer

3.3 Transfer the lysate to an appropriately sized RNase-free tube and homogenize using a rotorstator homogenizer at maximum speed for at least 00:00:45 .

Centrifuge the homogenate at ~ 2600 x g, 00:05:00 , then transfer the supernatant to a clean RNase-free tube.

5m 45s

Proceed to Binding, Washing, and Elution

13m 45s

4

Note

Follow the steps below to bind, wash, and elute the RNA from your sample:

Add one volume 70% ethanol to each volume of cell homogenate (prepare the sample as described in specific protocols.

Note

If part of the sample is lost during homogenization, adjust the volume of ethanol accordingly.

5 Vortex to mix thoroughly and to disperse any visible precipitate that may form after adding ethanol.

6 Transfer up to 700 µL of the sample (including any remaining precipitate) to the Spin Cartridge (with the Collection Tube).

7 Centrifuge at 12000 x g, 00:00:15 , Room temperature . 10m 15s

- Discard the flow-through, and reinsert the Spin Cartridge into the same collection tube.

Note

If you are processing the maximum starting amount of sample, you may centrifuge for up to 00:10:00 to completely pass the lysate through the Spin Cartridge.

8 Repeat Steps 6–7 until the entire sample is processed.

Note

Optional: If DNA-free total RNA is required, proceed to On-column PureLink® DNase Treatment Protocol.

9 Add 700 µL wash buffer I to the Spin Cartridge. 15s

- Centrifuge at 12000 x g, 00:00:15 , Room temperature . Discard the flow-through and the Collection Tube.
- Place the Spin Cartridge into a new Collection Tube.

10 Add 500 µL Wash Buffer II with ethanol to the Spin Cartridge.

11 Centrifuge at 12000 x g, 00:00:15 , Room temperature . 15s

- Discard the flow-through and reinsert the Spin Cartridge into the same collection tube.

12 Repeat Steps 10–11 once.

13 Centrifuge the Spin Cartridge at 12000 x g for 00:01:00 - 00:02:00 to dry the membrane with attached the RNA. Discard the collection tube and insert the Spin Cartridge into a recovery tube. 3m

14 Add 30 µL –3 × 100 µL RNase–Free Water to the center of the Spin Cartridge (see Elution Parameters).

> **Note**
>
> We diluted in 50 µL of water

15 Incubate at Room temperature for 00:01:00 . 1m

16 Centrifuge the Spin Cartridge for 00:02:00 at ≥ 12000 x g , Room temperature to elute the RNA from the membrane into the recovery tube. 2m

> **Note**
>
> If you are performing sequential elutions, collect all elutes into the same tube (see Elution Parameters).

17 Store your purified RNA or proceed to Analyzing RNA Yield and Quality or to DNase I Treatment after RNA purification.