Data sheet

HigherPurity™ Total RNA Extraction Kit

Cat. No: AN0280 (50 reactions)

Description

HigherPurity™ Total RNA Extraction Kit provides a rapid method for the extraction and purification of total RNA from cultured animal cells, tissue samples, blood, bacteria yeast, fungi, biologicals fluids and plants. The purification is based on spin column chromatography using a resin separation matrix.

Features

- **High yields**: up tp 50µg; depends on type of sample.
- **Ready to use** RNA.
- **Just a few minutes** (about 30-45 min).

Quality control

Each lot is tested in accordance to internal procedures.

Kit Storage:

HigherPurity™ Total RNA Extraction Kit can be stored at room temperature. The kit components are stable for 1 year, if stored properly.

Kit Components

Item	AN0280
RNA Buffer Lysis*	33ml
RNA Wash Buffer-1	35 ml
RNA Wash Buffer-2**	17 ml
RNA Elution Buffer	5ml
AF Solution (Antifoam)	1 ml
DNase I, RNase free (lyophilized)***	2 tubes
10x DNase I Reaction Buffer	5ml
RNAprep spin column	50
Collection Tubes	50

Note

* **OPTIONAL:** Before beginning the lysis and homogenization steps, prepare a fresh amount of RNA Buffer Lysis containing 1% 2-mercaptoethanol (β-ME) [Not included] for each purification procedure. Add 10 µL β-ME for each 1 mL RNA Buffer Lysis.
The use of β-ME in lysis is highly recommended for most animal tissues, particularly those know to have a high RNAse content (ex. pancreas), as well as for most plant tissues. Alternatively, the Lysis Buffer can be used as provided.

**Add the volume ethanol (96%-100%) specified [Not included] to RNA Wash Buffer prior to initial use (see bottle label for volume). After ethanol has been added, mark the bottle to indicate that this step has been completed.

*** Before using for the first time, reconstitute the DNase I lyophilizate in 255 µl of Nuclease-free water. Incubate 1 minute at room temperature. Mix carefully by inverting the tube several times. The Proteinase K solution can be stored for several days at 2–8 °C. For longer-term storage, the unused portion of the solution may be stored in aliquots at – 20 °C until needed.

For Research Use Only.
CAUTION: Not for human or animal therapeutic or diagnostic use.

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SAMPLE PREPARATION

A. FRESH OR FROZEN SOLID TISSUE

Quantity: 1-30 mg
Sample material: animal or human tissues.
Divide tissue into small fragments with tweezers and scissors or scalpel. Follow one of homogenization methods described below or go to **step 1** of the **RNA ISOLATION PROTOCOL**.

Liquid nitrogen, dry ice (LN2, CO2)

1. Put tissue frozen in LN2 or CO2 in a previously chilled, sterile mortar. Using a chilled piston, carefully, but firmly crush the tissue into smaller pieces and then, into a pulp.
2. Transfer the powder thus obtained into a 2 ml tube containing 600 µl **RNA Buffer Lysis** and **20 µl AF solution** and go to **step 2** of the **RNA ISOLATION PROTOCOL**.

Homogenization using a mechanical homogenizer equipped with knives

1. Place the tissue in a 2 ml tube, add 100 µl **RNA Buffer Lysis** and carefully homogenize with a sterile homogenizer tip.
2. After homogenization, retrieve the tissue remains from the knife tip by washing it with 500 µl **RNA Buffer** and **20 µl AF solution Lysis**. Combine the fractions thus obtained and transfer the entire volume to a new 2 ml tube.
3. Continue the isolation from **step 2** of the **RNA ISOLATION PROTOCOL**.

Homogenization using bead-beating tubes

1. Add 200 µl **RNA Buffer Lysis** and **20 µl AF solution** to a 2 ml ceramic bead-beating tube and suspend the sliced tissue in the buffer.
2. Homogenize by vortexing using the appropriate 2 ml tube adaptor for at least 5 min at maximum speed.
3. Add 400 µl **RNA Buffer Lysis** and mix by pipetting.
4. Continue the isolation from **step 2** of the **RNA ISOLATION PROTOCOL**.

B. CELL CULTURES

Quantity: 10^4-10^7 cells
Sample material: cell suspension or adherent cells, fresh or frozen at -80°C or 196°C.

1. Thaw frozen cells at 37°C. Centrifuge the cells suspended in growth medium or PBS buffer in a 15 ml falcon tube or a 1.5-2 ml Eppendorf tube at 3000 x g. If a compact cell pellet is not formed, wash the cells twice with 1 ml cold PBS buffer.
2. Add 600 µl **RNA Buffer Lysis** and **20 µl AF solution**. Mix thoroughly by vortexing for 30 s and subsequent pipetting. Transfer everything to a new 2 ml tube.
3. Continue the isolation from **step 2** of the **RNA ISOLATION PROTOCOL**.

FRESH AND FROZEN PLANT SAMPLES

Quantity: The maximum recommended input of plant tissue is 30 mg or 5 x 10^6 plant cells.
Sample material: Both fresh and frozen plant samples can be used for this protocol. Samples should be flash frozen in liquid nitrogen and transfer immediately to a -70ºC freezer for long-term storage. Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised.

1. Transfer the tissue into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the tissue thoroughly using a mortar and pestle. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
2. Transfer the sample to an RNase-free tube (not provided)) and add 600 µl **RNA Buffer Lysis** and **20 µl AF solution**.
3. Homogenize with an electrical homogenizer. Spin the lysate for 2 minutes to pellet any cell debris. Transfer de supernatant to another RNase-free microcentrifuge tube.
4. Continue the isolation from **step 2** of the **RNA ISOLATION PROTOCOL**.

BACTERIA CULTURE

Quantity: It is recommended that no more than 1 x 10^9 bacteria cells be used. Bacterial growth can be measured using a spectrophotometer, as a general rule, an *E. coli* culture containing 1 x 10^9 bacteria cells has an OD_{600} of

Prepare the appropriate lysozyme-containing TE buffer, this solution should be prepared with sterile, RNase free TE buffer, and kept on ice until needed. For Gram-negative the concentration of lysozyme will be of 1 mg/ml and for Gram-positive the concentration of lysozyme will be of 3 mg/ml.

1. Pellet bacteria by centrifugation at 14.000 x g for 1 minute.
2. Decant supernatant, and carefully remove any remaining media by aspiration. Resuspended the bacteria in
3. 100 µl of appropriate lysozyme-containing TE by vortexing. Incubate at room temperature for 10 minutes.
4. Add 300 µl of **RNA Buffer Lysis**. Lyse cells by vortexing for 15 seconds. Ensure that the mix become transparent before proceeding to the next step. Incubate 5 minutes at room temperature.
5. Continue the isolation from **step 2** of the **RNA ISOLATION PROTOCOL**.

RNA ISOLATION PROTOCOL

1. Place the biological material in a 2 ml tube. Add **600 µl** **RNA Buffer Lysis** and **20 µl** **AF solution** and vortex for 60 sec.
2. Centrifuge for 2 min at 15.000 x g.
3. Transfer the supernatant into a 1.5-2ml microcentrifuge tube. Add **600 µl** 70% ethanol to the transferred supernatant. Mix well by pipetting or vortexing.
4. Transfer up to **700 µl** of the obtained mixture into an **RNAprep spin column** placed in a **collection tube**. Centrifuge for 15 sec at 15.000x g. Discard the flow-through and reuse the column, together with the collection tube.
5. Transfer the remaining mixture into the same spin column and centrifuge at 15.000 x g for 15 sec. Discard the flow-through and place the spin column in a new collection tube.

DNA Removal Option:

a. Prewash the spin column with **500 µl** **RNA Wash Buffer-2** and centrifuge for 1 min at 15.000 x g.

b. For each isolation mix **90 µl** **10x DNase I Reaction Buffer** and **10 µl** reconstituted **DNase I**. Mix by inverting the tube.

c. Apply **95 µl** mixture onto the center of the spin column.

d. Incubate 15 minutes at room temperature.

e. Add **600 µl** **RNA Wash Buffer-1** and centrifuge for 20 sec at 15.000 x g. Discard the flow-through and reuse the collection tube and proceed to step 6.

6. Add **700 µl** **RNA Wash Buffer-1** and centrifuge for 15 sec at 15.000 x g. Discard the flow-through and reuse the collection tube.
7. Add **500 µl** **RNA Wash Buffer-2** and centrifuge for 30 s at 15.000 x g. Discard the flow-through and reuse the collection tube.
8. **Repeat Step 7.**
9. Centrifuge for 1-2 min at 15.000 x g. Discard the collection tube and flow-through and carefully transfer the spin column to a sterile 1.5 ml Eppendorf microcentrifuge tube.

The wash buffer contains alcohol, which may interfere with some enzymatic reactions and also decrease the elution efficiency. It is therefore vital to remove the alcohol completely from the spin column before elution.

10. Add **50-100 µl** **RNA Elution buffer** onto the centre of the spin column membrane.
11. Incubate the spin column at room temperature for 3 min. Centrifuge at 15.000 x g for 1 min.
12. Remove the spin column and place the tube with the eluted RNA in a freezing rack. The isolated RNA is ready for use in downstream applications or for storage at -80°C.

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