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DATA SHEET

Version: 02
Revision date: 30/05/2023

1. Identification

Product name **HigherPurity™ Total RNA Extraction Kit**

Cat. No **AN0280 (50 reactions)**
Cat. No **AN0280-XL (250 reactions)**

2. Description

Total RNA Extraction Kit provides a rapid method for the extraction and purification of total RNA from cultured animal cells and tissue samples. The purification is based on spin column chromatography using a resin separation matrix.

3. Composition

Item	Quantity	
	AN0280	AN0280-XL
RNA Buffer Lysis*	33 ml	165 ml
RNA Wash Buffer-1	35 ml	175 ml
RNA Wash Buffer-2**	17 ml	82 ml
RNA Elution Buffer	5 ml	5 x 5 ml
RNAprep spin column	50	5 x 50
Collection Tubes	50	5 x 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.
Protect the RNA Buffer Lysis and RNA Wash Buffer-1 from the sunlight!

4. Features

- High yields: up tp 50µg; depends on type of sample.
- Ready to use RNA.
- Just a few minutes (about 45-60 min).

5. Storage specifications

Total RNA Extraction Kit can be stored at room temperature.

6. Quality Control

Each lot is tested in accordance to internal procedures.

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7. Further information

Product Use Limitations	This product is developed, designed, and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Safety Information	When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at www.canvaxbio.com where you can find, view, and print the MSDS for each CANVAX kit.

8. 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 (LN_2, CO_2)

1. Put tissue frozen in LN_2 or CO_2 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 go to step 2 of the RNA ISOLATION PROTOCOL.

After pulping, a thin, sticky layer may be formed, rather than a powder. If this occurs, add 600 µl RNA Buffer Lysis to a mortar and reconstitute the tissue by pipetting and then transfer the lysate into a sterile RNase-free 2 ml tube. Remember to retrieve a tissue remains from the piston as well.

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.

Optional: in order to avoid foaming, antifoam agent can be added. (Not included)

2. After homogenization, retrieve the tissue remains from the knife tip by washing it with 500 µl RNA Buffer. 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 (Not included)

1. Add 600 µl RNA Buffer Lysis to a 2 ml ceramic bead-beating tube and suspend the sliced tissue in the buffer.

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Optional: in order to avoid foaming, antifoam agent can be added. (Not included)

2. Place the tube in a tissue homogenizer and homogenize for 30–60 s at 3000–4000 x g. If necessary, repeat the procedure.

If the tissue homogenizer is not available, the tissue may be homogenized by vortexing with the use of an appropriate 2 ml tube adaptor for at least 5 min at maximum speed.

3. Continue the isolation from step 2 of the RNA ISOLATION PROTOCOL

B. CELL CULTURES

Quantity: up to 10^7 cells

Sample material: cell suspension or adherent cells, fresh or frozen.

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 400 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 mix by vortexing.

3. Continue the isolation from step 2 of the RNA ISOLATION PROTOCOL.

PRIOR TO ISOLATION

1. Mix well each buffer supplied with the kit. Do not mix RNA Buffer Lysis vigorously.

2. Ensure that ethanol has been added to RNA Wash Buffer-2.

3. Prepare 70% ethanol using DEPC-treated water.

4. Examine RNA Buffer Lysis and RNA Wash Buffer-1. If a sediment occurred in any of them, incubate it at 50˚C (Buffer Lysis) or at 37˚C (Wash Buffer-1) mixing occasionally until the sediment has dissolved.

Cool to room temperature.

RNA ISOLATION PROTOCOL

1. Place the biological material in a 2 ml tube. Add 600 µl RNA Buffer Lysis 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.

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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 RNAprep spin column and centrifuge at 15.000 x g for 15 sec. Discard the flow-through and place the RNAprep spin column in a new collection tube.

OPTIONAL (DNase treatment)

a. Prewash the RNAprep spin column with 500 µl RNA Wash Buffer-2 and centrifuge for 1 min at 15.000 x g. Discard the filtrate and reuse the collection tube.

b. For each isolation mix 90 µl DNase I Reaction Buffer and 10 µl reconstituted DNase I (Not included in the kit). Mix by inverting the tube.

c. Apply DNase I plus Reaction Buffer onto the center of the RNAprep spin column.

d. Incubate 5 minutes at room temperature.

e. Add 600 µl RNA Wash Buffer-1 and centrifuge for 15 sec at 15.000 x g. Discard the flow-through and reuse the collection tube and proceed to step 7.

6. (Omit after DNase treatment) 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 15 sec at 15.000 x g. Discard the flow-through and reuse the collection tube.

8. Repeat Step 7.

9. Centrifuge for 90 sec at 15.000 x g. Discard the collection tube and flow-through and carefully transfer the spin column to a sterile 1.5 ml RNase-free microcentrifuge tube.

The wash buffer-2 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. Centrifuge at 15.000 x g for 2 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