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DATA SHEET

Version: 03
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1. Identification

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

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

2. Description

HigherPurity™ Total RNA Extraction Kit provides a rapid method for the extraction and purification of total RNA from animal cells, animal tissues, bacteria, yeast, paraffin fixed sample, fungi and for RNA clean-up. The purification is based on spin column chromatography using a resin separation matrix.

3. Composition

Items	Quantity	
	AN0280 (50 rxn)	AN0280-XL (250 rxn)
RNA Buffer Lysis *	20 ml	100 ml
RNA Wash Buffer 1	27 ml	135 ml
RNA Wash Buffer 2 ** (WB2)	16 ml	80 ml
RNase-free ddH2O	5 ml	15 ml
RNAprep Spin Columns	50	250
Filter Column	50	250
Collection tube (2mL)	100	500
1.5 ml microcentrifuge tube	50	250
Micropestle	50	250

Note

* 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 uses of this kit, (excluding RNA cleanup), and particularly those know to have a high RNAse content (ex. pancreas). Alternatively, the RNA Buffer Lysis can be used as provided.

**Add the volume ethanol (96%-100%) specified [Not included] to RNA Wash Buffer 2 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.

! Caution: Please wear gloves when using these products. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water.

! Caution: RNA Buffer Lysis and Wash Buffer 1 contain guanidinium salts which can form highly reactive compounds when combined with bleach. DO NOT add bleach or acidic solutions directly to the preparation waste.

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4. Features

- High quality of Total RNA obtained.
- High yields: up to 60µg; depends on type of sample.
- Safe: avoids the use of harmful chemicals as phenol or chloroform.
- Easy and fast protocol: results in 30-45 minutes.
- Pure RNA: ready-to-use in all downstream applications.

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.

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. GENERAL PROTOCOL. ANIMAL CELLS

Additional Reagents: ß-Mercaptoethanol, RNase-free 70% etanol

1. Collect between 1 and 5×10^6 cells by centrifuging at 300 xg for 5 minutes at 4˚C. Carefully remove and discard the supernatant.
   Note: Avoid overloading the tube; too many cells may lead to incomplete lysis and, subsequently, a lower RNA yield and purity.
2. To the cell pellet, add 350 µl of RNA Buffer Lysis and 3.5 µl of ß-Mercaptoethanol. Vortex the mixture vigorously for 1 minute to ensure the cells are fully resuspended.
   Note: If clumps remain after vortexing, gently pipette the mixture up and down to break them apart.
3. Place a Filter Column onto a Collection Tube and carefully transfer the lysate into the Filter Column. Centrifuge the assembly at 18,000 xg for 2 minutes.
4. Transfer the clarified supernatant from the Collection Tube into a new microcentrifuge tube (tube not provided) and note the volume. Discard the Filter Column and the original Collection Tube.
   Note: When transferring, avoid aspirating any debris or pellet remnants.
5. Add an equal volume of RNase-free 70% ethanol to the supernatant and mix thoroughly by vortexing.

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6. Set an RNAprep Spin Column on a Collection Tube and transfer the ethanol-mixed sample (including any precipitate) onto the column.

- Centrifuge:
  Centrifuge the column at 18,000 xg for 30 seconds. Discard the flow-through and return the RNAprep Spin Column to the Collection Tube.

- Vacuum:
  Apply a vacuum at -6 inches Hg until the column is fully emptied. Then, switch off the vacuum and release the pressure from the manifold.

7. **Optional Step: On-Column DNase I Digestion**

To remove any genomic DNA contamination, perform the following steps. Otherwise, proceed directly to step 8.

7a. Add 250 µl of RNA Wash Buffer 1 onto the RNAprep Spin Column.
- Centrifuge:
  Spin at 18,000 xg for 30 seconds, discard the flow-through, and reposition the column into the Collection Tube.
- Vacuum:
  Apply vacuum at -6 inches Hg for 1 minute, then turn off the vacuum and release the pressure.

7b. Add 750 µl of RNase-free 70% ethanol to the RNAprep Spin Column.
- Centrifuge:
  Spin at 18,000 xg for 30 seconds, discard the flow-through, and return the column to the Collection Tube.
- Vacuum:
  Apply vacuum at -6 inches Hg for 1 minute, then switch off and release the pressure.

7c. Dispense 50 µl of RNase-free DNase I solution (0.25 U/µl, not provided) directly onto the center of the membrane in the RNAprep Spin Column. Allow the column to stand at room temperature for 15 minutes.

7d. Add 250 µl of RNA Wash Buffer 1 to the column.
- Centrifuge:
  Spin at 18,000 xg for 30 seconds, discard the flow-through, and place the column back into the Collection Tube.
- Vacuum:
  Apply vacuum at -6 inches Hg until the column is completely emptied, then turn off the vacuum and release the pressure.

7e. After DNase I treatment, proceed directly to step 9.

8. Add 500 µl of RNA Wash Buffer 1 to the RNAprep Spin Column.

- Centrifuge :
  Spin at 18,000 xg for 30 seconds. Discard the flow-through and reposition the RNAprep Spin Column into the Collection Tube.

- Vacuum:
  Apply a vacuum at -6 inches Hg until the column is completely emptied, then switch off the vacuum and release the pressure.

9. Add 750 µl of RNA Wash Buffer 2 to the RNAprep Spin Column.

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- Centrifuge:
  Spin at 18,000 xg for 30 seconds. Discard the flow-through and return the RNAprep Spin Column to the Collection Tube.

- Vacuum:
  Apply vacuum at -6 inches Hg until the column is fully emptied, then switch off the vacuum and release the pressure.

  Note: Ensure that ethanol has been added to RNA Wash Buffer 2 during its initial use.

10. Repeat step 9 for an additional wash.

11. Dry the Column by centrifuging the RNAprep Spin Column at 18,000 xg for 3 minutes. Discard the flow-through and return the column to the Collection Tube.
    Important: This drying step prevents residual wash buffer from inhibiting subsequent enzymatic reactions.

12. Place the RNAprep Spin Column onto a microcentrifuge tube (provided).

13. Add 30–50 µl of RNase-free ddH_2O directly onto the center of the column membrane. Allow the column to stand at room temperature for 1 minute.
    Important: Ensure that the RNase-free water is dispensed directly onto the membrane and is completely absorbed.
    Note: Using less than 30 µl may lower the RNA yield.

14. Centrifuge the RNAprep Spin Column at 18,000 xg for 30 seconds to elute the RNA. Store the eluted RNA at -70˚C.

B. FRESH OR FROZEN SOLID TISSUE

Quantity: Up to 30 mg
Sample Material: Tissue sample
Additional Equipment: Liquid nitrogen, mortar, rotor-stator homogenizer or 20-G needle syringe, ß-Mercaptoethanol, RNase-free 70% ethanol

Procedure A: Homogenization via Rotor-Stator Homogenizer or 20-G Needle Syringe

1. Weigh no more than 30 mg of tissue. While keeping the sample frozen with liquid nitrogen, use a pre-cooled mortar to grind the tissue until a fine powder is achieved. Immediately transfer this powder into a sterile microcentrifuge tube (tube not provided).
   Note: Ensure the sample does not thaw during weighing and grinding.

2. Add 350 µl of RNA Buffer Lysis and 3.5 µl of ß-Mercaptoethanol to the powder. Homogenize the mixture either by operating a rotor-stator homogenizer or by passing the lysate through a 20-G needle syringe 10 times. Then, incubate the sample at room temperature for 5 minutes.
   Important: For more resilient tissues, using an appropriate homogenization device (e.g., a rotor-stator homogenizer) is recommended to optimize RNA release.

3. Proceed with the Animal Cells Protocol starting at step 3.

Procedure B: Alternative Method Using a Micropestle

1. Place up to 30 mg of tissue in a microcentrifuge tube. Introduce 350 µl of RNA Buffer Lysis and 3.5 µl of ß-Mercaptoethanol, then use the provided micropestle to thoroughly grind the tissue.

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2. Pass the resulting mixture through a 20-G needle syringe between 10 and 20 times. Allow the sample to sit at room temperature for 5 minutes.
   Note: For tissues with lower cell density or that are harder to break down, the method in Procedure A is advisable.
3. Continue with the Animal Cells Protocol beginning at step 3.

C. BACTERIA

Additional requirements:

- ß-Mercaptoethanol
- RNase-free 70% ethanol
- 30˚C water bath or heating block
- 2 ml screw centrifuge tube
- Lysozyme reaction solution (10 mg/ml lysozyme; 20 mM Tris-HCl, pH 8.0; 2 mM EDTA; 1.2% Triton)
- Acid-washed glass beads (500–700 µm)

Quantity: up to 10^7 cells

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

1. Transfer up to $1×10^9$ well-grown bacterial cells from culture into a 2 ml screw centrifuge tube.
   Note: Ensure that the total RNA harvested does not exceed the column's binding capacity (100 µg) when estimating the sample size. Using too many cells may result in incomplete cell lysis and lower RNA yield and purity. If the RNA content is hard to predict for some species, use no more than $5×10^8$ cells as your starting sample.
2. Pellet the bacterial cells by centrifuging at 18,000 xg for 2 minutes at 4˚C. Discard all the supernatant.
3. Add 100 µl of the lysozyme reaction solution to the pellet. Pipette up and down to completely resuspend the cells, then incubate the tube at 37˚C for 10 minutes.
4. Add 350 µl of RNA Buffer Lysis and 3.5 µl of ß-Mercaptoethanol to the suspension.
5. Introduce 250 mg of acid-washed glass beads (500–700 µm) and vortex vigorously for 5 minutes to disrupt the cells.
6. Centrifuge the mixture at 18,000 xg for 2 minutes to sediment insoluble material. Carefully transfer the supernatant to a new microcentrifuge tube (tube not provided) and record the volume.
   Note: Avoid transferring any debris or pellet material.
7. Follow the Animal Cells Protocol starting from step 5.

D. YEAST

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Additional requirements:
• ß-Mercaptoethanol
• RNase-free 70% ethanol

For enzymatic disruption:
• Lyticase or zymolase
• Sorbitol buffer (1 M sorbitol; 100 mM EDTA; 0.1% ß-ME)
• 30˚C water bath or heating block

For mechanical disruption:
• 2 ml screw centrifuge tube
• Acid-washed glass beads (500–700 µm)

1. Collect up to 5×10^7 yeast cells by centrifuging at 5,000 xg for 10 minutes at 4˚C. Discard all of the supernatant.
2. Disruption

2A. Enzymatic Disruption:

2A-1. Resuspend the cell pellet in 600 µl of sorbitol buffer (not provided). Add 200 U of zymolyase (or lyticase) and incubate at 30˚C for 30 minutes.
Note: Prepare the sorbitol buffer immediately before use.
2A-2. Centrifuge at 300 xg for 5 minutes to pellet the spheroplasts. Discard the supernatant.
2A-3. Add 350 µl of RNA Buffer Lysis and 3.5 µl of ß-Mercaptoethanol to the pellet. Vortex vigorously for 1 minute to disrupt the spheroplasts, then allow the mixture to stand at room temperature for 5 minutes.

2B. Mechanical Disruption:

2B-1. Add 350 µl of RNA Buffer Lysis and 3.5 µl of ß-Mercaptoethanol to the cell pellet, and vortex thoroughly to ensure complete resuspension.
2B-2. Transfer the resuspended cells to a 2 ml screw centrifuge tube. Add 250 mg of acid-washed glass beads (500–700 µm) and vortex vigorously for 15 minutes to achieve complete cell disruption.

3. Follow the Animal Cells Protocol starting from step 5.

D. PARAFFIN-EMBEDDED TISSUE

Additional equipment:
• xylene and ethanol (96–100%)
• liquid nitrogen and a mortar
• a rotor-stator homogenizer or 20-G needle syringe
• ß-Mercaptoethanol
• RNase-free 70% ethanol

1. Transfer up to 15 mg of paraffin-embedded tissue into a microcentrifuge tube (not provided). Remove any excess paraffin to reduce the size of the tissue slice.

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2. Add 0.5 ml of xylene, mix thoroughly, and incubate at room temperature for 10 minutes.
3. Centrifuge at full speed for 3 minutes, then carefully pipette off the supernatant.
4. Add 0.25 ml of xylene, mix well, and incubate at room temperature for 3 minutes.
5. Centrifuge at full speed for 3 minutes, and remove the supernatant by pipetting.
6. Repeat steps 4 and 5.
7. Add 0.3 ml of ethanol (96–100%) to the deparaffinized tissue, gently vortex to mix, and incubate at room temperature for 3 minutes.
8. Centrifuge at full speed for 3 minutes, then remove the supernatant by pipetting.
9. Repeat steps 7 and 8.
10. Proceed with the Animal Tissue Protocol starting from step 1 for sample disruption, then continue with the Animal Cells Protocol beginning at step 3.

E. RNA CLEAN UP

Additional equipment:
• ethanol (96–100%)

1. Transfer 100 µl of the RNA sample into a microcentrifuge tube (not provided). If the sample volume is less than 100 µl, add RNase-free water to bring it to 100 µl.
2. Add 300 µl of RNA Buffer Lysis and 300 µl of RNase-free ethanol (96–100%), then vortex thoroughly to mix.
3. Place an RNAprep Spin Column into a Collection Tube, and transfer the ethanol-treated sample mixture onto the column. Centrifuge at 18,000 xg for 1 minute, discard the flow-through, and return the RNAprep Spin Column to the Collection Tube.
4. Continue with the Animal Cells Protocol starting from step 8.