Cell & Tissue RNA Extraction Kit

Catalog Number: EXNA077
Size: 50 Tests

For Research Use Only. Not Intended for Diagnostic or Therapeutic Use.

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Product Description

The Cell & Tissue RNA Extraction Kit uses a rapid, phenol-free and chloroform-free RNA extraction technique for efficient extraction of total RNA from animal tissue and cells. This kit utilizes a lysis buffer that rapidly lyses cells and inactivates RNase, without the need for β-mercaptoethanol commonly used in conventional kits. The genomic DNA removal column effectively removes gDNA residue, eliminating the need for DNase digestion and allowing direct use of RNA for tests such as RT-PCR and qPCR. If downstream experiments are highly sensitive to trace DNA contamination, additional DNase treatment can be employed.

This kit efficiently extracts high-purity RNA in under 30 minutes.

Limitations

- For Research Use Only. Not Intended for Diagnostic or Therapeutic Use.

Precautions

- Always wear appropriate protective clothing and follow safe laboratory procedures.
- Ensure that equipment used in the experiment is RNase-free, including gloves, pipette tips, tubes, etc.
- RNase removal may be achieved by baking glassware at 150˚C for 4 hours. Likewise, soak plasticware in 0.5M NaOH for 10 minutes and wash it thoroughly with water.
- Low temperature storage may cause precipitation. Gently heat in a water bath at 37˚C.
- The sample processing volume should not exceed the processing capacity of the genomic DNA removal column and RNA adsorption column to avoid DNA residue or yield reduction. The RNA/DNA of various samples differs greatly. Therefore, limit cell samples to 3-4×10^6 cells and tissue samples to 10 mg when testing new samples. Optimize the processing volume for subsequent experiments based on the results.
- If residues are caused by excessive DNA content or rigorous fluorescent quantitative PCR is required, it is recommended to select intron-spanning primers or treat the RNA extract with RNase-free DNase.

Materials Provided & Storage

Shipped at ambient temperature.
Store the kit at 2-30˚C for up to 12 months**. Protected from light.**

Component	Size	Quantity
RLT Lysis Buffer	50 mL	1 Bottle
Wash Buffer 1	40 mL	1 Bottle
Wash Buffer 2*	10 mL	1 Bottle
70% Ethanol*	9 mL	1 Bottle
Eluent	10 mL	1 Bottle
Removal Column and Collection Tube	50 Pcs	1 Pack
Adsorption Column and Collection Tube	50 Pcs	1 Pack

***See Preparation of Reagents for final dilution instructions.**

Preparation of Reagents

Preparation of Wash Buffer 2: Add 42 mL ethanol (96-100%) to the Wash Buffer 2 reagent bottle before use.
Preparation of 70% Ethanol: Add 21 mL ethanol (96-100%) to the 70% Ethanol reagent bottle before use.

Protocol

Cultured Cell Preparation:

1. Collect ≤ 1×10^7 cells by centrifuging at 300 x g for 5 minutes or 13,000 x g for 10 seconds, and remove supernatant.
2. Add 350 µL (< 5×10^6 cells) or 600 µL (5×10^6 ~1×10^7 cells) of RLT Lysis Buffer, mix well by pipetting up and down, shake vigorously by hand for 20 seconds. Place the Removal Column into the Collection Tube and add all the lysis mixture to the Removal Column.
3. Proceed to Extraction Protocol.

Animal Tissue Preparation:

1. Samples may be homogenized by either of the following methods:
   a. **Electric homogenization**: Quickly cut fresh tissues into small pieces with a scalpel, add 350 µL (<20 mg tissue) or 600 µL (20 mg-30 mg tissue) of RLT Lysis Buffer, and then electrically homogenize the mixture thoroughly for 20-40 seconds.
   b. **Grinding with liquid nitrogen:** Grind the tissue into fine powder with liquid nitrogen, transfer an appropriate amount of fine tissue powder (20 mg/30 mg) into an RNase-free 1.5 mL centrifuge tube containing 350 µL (20 mg tissue) or 600 µL (30 mg tissue) of RLT Lysis Buffer, and shake the tube vigorously by hand for 20 seconds to fully lyse.
   Optional Step: Use a disposable 1 mL (with a 0.9 mm needle) syringe with a blunt needle to vigorously push and pull the plunger at least 10 times until a satisfactory homogenization effect is obtained, to shear DNA and reduce viscosity to prevent column plugging and improve yield.
2. Centrifuge the homogenized mixture at 13,000 x g for 3 minutes. Place the Removal Column into the Collection Tube and add all the lysis mixture to the Removal Column.
3. Proceed to Extraction Protocol.

Extraction Protocol:

1. Centrifuge at 13,000 x g for 1 minute and retain the filtrate. **Note:** *ensure that all liquid is filtered after centrifugation. Increase the centrifugal force and time if necessary.*
2. Accurately measure the volume of the filtrate with a pipette, and an equal volume of 70% Ethanol (prepared per Preparation of Reagents) and mix well by pipetting up and down.
3. Immediately transfer the mixture to the Adsorption Column, which has been placed into the Collection Tube, centrifuge at 13,000×g for 30 seconds and discard the filtrate. **Note:** *do not transfer more than 700 µL at a time. If more than 700 µL is required, divide the sample and perform two separate transfers and centrifugation steps.*
4. Add 700 µL of Wash Buffer 1, place at room temperature for 1 minute, centrifuge at 12,000 x g for 30 seconds, and discard the filtrate.
5. Add 500 µL of Wash Buffer 2 (prepared per Preparation of Reagents) , centrifuge at 12,000 x g for 30 seconds, and discard the filtrate.
6. Repeat Step 5.
7. Place the Adsorption Column back into the Collection Tube and centrifuge at 13,000 x g for 2 minutes to completely remove residual ethanol via spin-drying. **Note:** *The presence of residual ethanol can inhibit downstream reactions.*
8. Place the Adsorption Column into an RNase-free 1.5 mL centrifuge tube, add 30 µL-50 µL of Eluent to the center of the adsorption column membrane, allow it to stand for 1 minute, and centrifuge at 12,000 x g for 1 minute. **Note:** *The yield can be improved by preheating the Eluent in a water bath at 50°-70°C. If the expected RNA yield exceeds 30 µg, pipette the eluted RNA back into the adsorption column and repeat Step 8; or add another 30 µL-50 µL of Eluent and repeat Step 8 and mix the two eluted RNA samples.*

Note: For samples with excessively high DNA content, Step 4 can be performed as follows:

a) Add 350 µL of wash buffer 1 to the Adsorption Column (placed into Collection Tube), centrifuge at 12,000 x g for 30 seconds, and discard the filtrate.
b) Add an appropriate amount of DNase working solution to the center of the Adsorption Column and place it at room temperature for 15 minutes.
c) Add 350 µL of wash buffer 1 to the Adsorption Column (placed into Collection Tube, centrifuge at 12,000 x g for 1 minute, and discard the filtrate.

Protocol Flowchart