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FOREGENE
EXPERT IN NUCLEIC ACID EXTRACTION

Cell Total RNA Isolation Kit

Cat.No.RE-03111/03112/03113

For total RNA purification from cultured cells
$10^4 \leq$ Cultured Cells $\leq 10^6$ (96/24/12/6-well plates)

Product introduction

This kit uses the spin column and formula developed by Foregene, which can efficiently extract high-purity and high-quality total RNA from cells cultured in 96, 24, 12, and 6-well plates. The kit provides an efficient DNA-Cleaning Column, which can easily separate the supernatant and cell lysate, bind and remove genomic DNA. The operation is simple and time-saving; the RNA-only Column can efficiently bind RNA with a unique formula. A large number of samples can be processed simultaneously.

The whole system is RNase-Free, so that the purified RNA is not degraded; the Buffer RW1, Buffer RW2 buffer washing system guaratee the obtained RNA free of protein, DNA, ion, and organic compound pollution.

Features&advantages:

- The whole process is operated at room temperature (15-25℃), **without ice bath and low temperature centrifugation.**
- The whole kit is RNase-Free, no need to worry about RNA degradation.
- DNA-Cleaning Column specifically binds DNA, so that the kit can remove genomic DNA contamination without adding additional DNase.
- High RNA yield: RNA-only Column and unique formula can efficiently purify RNA.
- Fast speed: easy to operate and can be completed in 11 minutes.
- Safety: No organic reagent is required.
- High quality: The purified RNA is of high purity, free of protein and other impurities, and can meet various subsequent experiments.

Kit application:

It is suitable for extraction and purification of total RNA from cultured cells in 96, 24, 12, and 6-well plates.

Application of RNA

The total RNA extracted from the Cell Total RNA Isolation Kit can be used for a variety of downstream molecular experiments such as cDNA synthesis, RT-PCR, Real Time PCR, Northern Blot, Dot Blot, in vitro translation, chip analysis, PolyA screening, molecular cloning, and RNase protection analysis.

Storage of RNA

It is recommended to use RNase-Free ddH_2O to elute RNA for immediate use in downstream experiments or store at -80°C. RNA can be stored for one year at -80°C storage.

Product quality control

According to the FOREGENE's Total Quality Management System, each batch of Cell Total RNA Isolation kits is rigorously tested multiple times to ensure the reliability and stability of the quality of each batch of kits.

Kit component

Kit composition	RE-03111	RE-03114
	50 T	200 T
Buffer cRL1*	25 mL	100 mL
Buffer cRL2	15 mL	60 mL
Buffer RW1*	25 mL	100 mL
Buffer RW2	24 mL	96 mL
RNase-Free ddH_2O	10 mL	40 mL
RNA-Only Column	50	200
DNA-Cleaning Column	50	200
Instruction	1	1

*Please wear gloves and take protective measures during the operation as Buffer cRL1 and Buffer RW1 contain irritating chaotropic salts.

Product Information

Format	Spin column	Purification component	Foregene column, reagent
Flux	1-24 samples	Time per prep	~11 min (24 samples)
Centrifuge	Desk centrifuge	Pyrolysis separation	Centrifugal separation
Sample	Cultured cell	Samples amount	10^4 -10^6
Elution volume	20-100 μL	Maximum loading volume	850 μL

Storage

This kit can be stored for 24 months at room temperature (15-25℃) and in dry environment; For further storage, please place it in 2-8℃ environment. Buffer cRL1 can be stored for a month at 4℃ after adding 2-hydroxy-1-ethanethiol (optional).

Note: If it is stored at a low temperature, it will crystallize easily. Please place the kit solution at room temperature (15-25°C) for a few minutes before use, or preheat it in a 37°C water bath for 10 minutes before use to dissolve the crystals.

Kit component information

◆ Buffer cRL1: Provides the environment required for cell lysis.

◆ Buffer cRL2: Provides a specific upper column environment for RNA.

◆ Buffer RW1: Removes impurities such as proteins and DNA from RNA.

◆ Buffer RW2: Remove residual salt ions from RNA.

◆ RNase-Free ddH_2O: Total RNA on elution of the purified column membrane.

◆ DNA-Cleaning Column: Specifically adsorbs DNA in cell lysates and filters to remove solid impurities from the lysates.

◆ RNA-only Column: Total RNA specifically adsorbed through the DNA-Cleaning Column filtrate.

DNA-Cleaning Column specifications

Mechanism	Specifically adsorp DNA
Function	Remove DNA contamination, Filter and separates the lysate
Maximum loading volume	800μl

RNA-Only Column specifications

Maximum RNA binding capacity	60μg
Maximum loading volume	700μl
RNA size distribution	RNA≥200nt
Minimum elution volume 1*	20μl
Selection of samples	Cultured cell
Maximum amount of starting material 2*	10^6 cells

1*: The minimum elution system of 20 μl is a reasonably recommended volume given for RNA recovery and concentration when the cell volume is small. If in order to increase the yield of RNA, the elution volume can be increased appropriately, such as using an elution system of 30-50 μl, in order to obtain it

2*: For larger sample sizes, use the Animal Total RNA Isolation Kit.

RNA yield and purity

RNA purified using Cell Total RNA Isolation Kit yields is related to the initial cell volume, freshness, cell retention time, and manipulation. The following is the extraction of RNA using this kit, the yield and purity of RNA in several cultured cells, in practice, may be slightly different from this data.

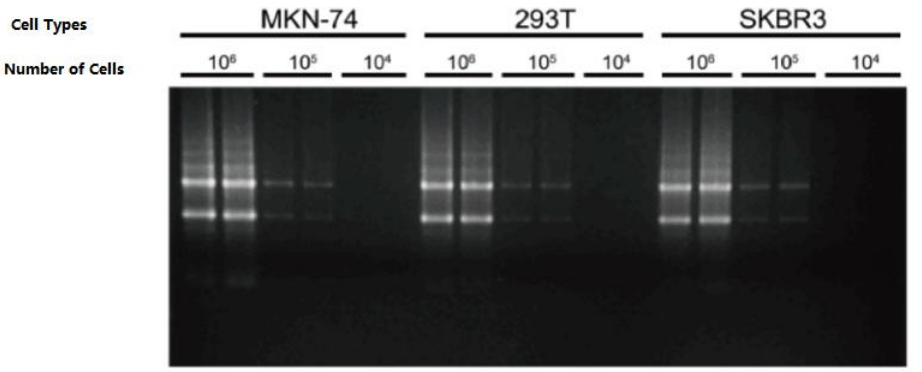
Cell Type	RNA yield (10^6)	OD260/280	OD260/230
MKN-74	15-20μg	1.8-2.1	1.8-2.1
293T	14-18μg	1.8-2.1	1.8-2.1
SKBR3	15-20μg	1.8-2.1	1.8-2.1

Note: The silica gel membrane will adsorb a small amount of liquid, and the volume of RNA product obtained after elution will be deviated.

RNA completeness

The quality of the RNA can be analyzed under ultraviolet light after denaturing agarose gel electrophoresis, ethidium bromide staining. On the gel, the band pattern of the ribosomal RNA should be obvious and clear. If the ribosomal RNA of any lane or a particular sample band shows inconspicuous, unclear, diffuse into small fragments of RNA, or disappears, it may be that the sample has undergone RNA degradation prior to processing or caused RNA degradation during purification.

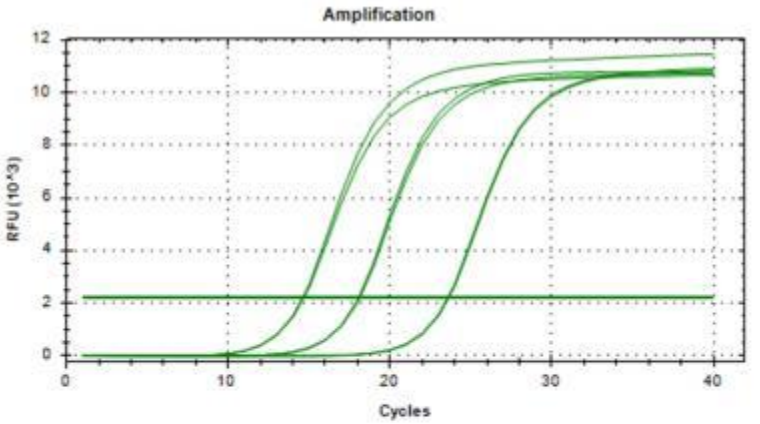
The figure below shows the RNA electrophoresis map obtained by the Foregene Cell Total RNA Isolation Kit processing different numbers of cell samples.



The agarose gel battery diagram of Cell Total RNA Isolation Kit treated the above different numbers of cells,20μl volume elution, take 2μl purified total RNA 1%.

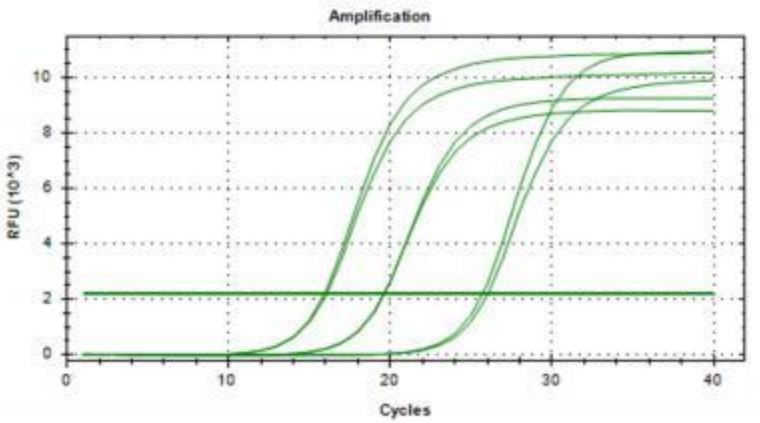
RT-qPCR graph of RNA

The Cell Total RNA Isolation kit improves the quality and stability of total RNA by RT-qPCR. The figure below shows the Foregene Cell Total RNA Isolation kit to extract the total RNA of different cells, and after reverse transcription, the qPCR pattern of gene A is detected.



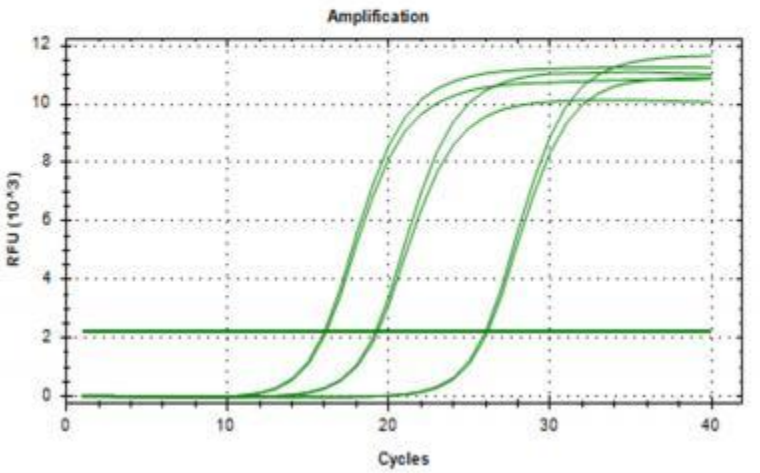
细胞类型：MKN-74

细胞数量：10^6、10^5、10^4



细胞类型：293T

细胞数量：10^6、10^5、10^4



细胞类型：SKBR3

细胞数量：10^6、10^5、10^4

Note：（Be sure to read the notes carefully before using）

- All procedures are carried out at room temperature(15-25℃), including centrifugation. **Do not use ice bath or centrifuge at low temperature (4°C).**
- The sample should avoid repeated freezing and thawing, otherwise it will lead to the degradation of the extracted RNA and the yield of RNA will also decrease.
- The yield and quality of RNA is tightly related to sample size and volume of elution. For every 500 μL of buffer cRL1, the recommended maximum cell volume is 10^6.
- Before using the kit, please add 2-hydroxy-1-ethanethiol to Buffer cRL1 (10 μL 2-hydroxy-1-ethanethiol per 1 mL Buffer RL1). Buffer cRL1 can be stored at 4℃ for 1 month after adding 2-hydroxy-1-ethanethiol. If the extracted RNA is not used to clone full-length cDNA, but only used for other downstream operations such as qPCR or sequencing analysis, it is not necessary to add 2-hydroxy-1-ethanethiol, and the result will not be affected.
- Before using the kit, please add anhydrous ethanol to buffer cRL2 and Buffer RW2 referring to the label on the reagent bottle for the dosage.
- The volume of elution should be no less than 20 μL, otherwise it will influence RNA yield.
- Please check if there is any precipitate in Buffer cRL1 and Buffer RW1. If the precipitate can be seen after storage at low temperature, the solution should be placed at room temperature(15-25℃) or 37℃ for a period of time. Use the solution after dissolving and blending.

Preparations before operation

Be sure to read the instructions carefully before using this kit. The Cell Total RNA Isolation Kit is simple, easy, and fast, and the instructions provide the correct way to use the entire kit. Please prepare the necessary experimental materials and equipment before use.

Experimental materials and equipment

- 10^4-10^6 cultured animal cells.
- 1.5ml RNase-Free EP tube.
- Tabletop centrifuges (≥ 13,400 ×g), pipettes, etc.

Self-prepared reagents

- Absolute ethanol
- β-Mercaptoethanol (optional)

Security

This product is for scientific research purposes only, do not use in medicine, clinical medical, food and cosmetics and other purposes.

When using the kit, wear lab coats, disposable latex gloves, disposable masks, etc. to protect yourself; and minimize human-introduced RNase contamination.

- Buffer cRL1 contains ionosy salts: denaturants, irritating.
- Buffer cRL2 contains absolute ethanol: flammable.
- Buffer RW1 contains ionosy salts: denaturants, irritating.
- Buffer RW2 contains absolute ethanol: flammable.

Operation Guide

Please select the appropriate lysis method for cell lysis according to your sample material. **The kit should be operated at room temperature (15-25° C) throughout the course, without ice bathing and centrifugation at low temperature.**

Sample selection and storage

The selection and preservation of samples largely determines the yield of RNA. RNA extraction should be performed using fresh animal tissues or cells whenever possible.

RNA degrades quickly after sample collection; if the collected sample is too late to extract the RNA, store it properly as soon as possible. We recommend that newly acquired samples should be immediately quick-frozen in liquid nitrogen, after which they should be stored at -80°C for a long time and avoid repeated freeze-thawing of the samples, or immediately soak the samples in RNA stabilizer RNAlater solution. To avoid degradation of RNA, sample collection and preservation should be carried out as quickly as possible.

Samples are saved in Buffer cRL1

RNA in Buffer cRL1 will not be degraded by RNase, if the cells added to Buffer cRL1 lysis if not immediately used, can be stored at room temperature for about 24h, stored at 4 °C for about 1 week, stored for a longer time at -80 °C, when using the solution at room temperature or 37 °C can be dissolved.

Initial sample dosage

The correct initial sample throughput is necessary for optimal RNA yield and purity, and the maximum sample throughput is related to the following factors:

- The type of sample itself and the abundance of the sample RNA;
- The amount of Buffer cRL1 determines the effective lysis of the sample;
- RNA-Only Column's RNA binding capacity.

Based on the above factors, we recommend that the initial amount of cells should not exceed 10^6, referring to the RNA yield table of 10^6 cell samples. If the sample is used in too much, Buffer cRL1 is not completely lyticed to the cells, resulting in low purity of the RNA obtained by purification; at the same time, the maximum carrying capacity of RNA-Only Column may be exceeded and precious samples may be wasted.

Cell lysis

Under Buffer cRL1 conditions, repeatedly pipette the mixed cells using a pipette. Sample fragmentation should be thorough until no cell mass is visible to completely destroy the cell membrane and organelles to release RNA, otherwise it will affect the yield of RNA and the next step of lysate separation (see procedure step 2) is prone to spin column blockage.

Material access instructions

Culture cells: Treat cells in a single treatment, do not exceed 10^6.

Prevent cross-contamination between samples

In order to avoid cross-contamination between samples, after each sampling, the edge of the sampling equipment or the part in direct contact with the sample needs to be immersed in a 2% sodium hypochlorite solution, repeatedly washed several times for cleaning, and then wiped the residual liquid with a clean paper towel before use. In order to facilitate the test, multiple sampling equipment can also be prepared and uniformly cleaned after use to ensure that each individual sample is used without contamination.

RNase contamination prevention

◆ Human contact is an important source of RNase contamination and some reagents may have a pungent odor, so change gloves frequently and wear disposable masks during operation.

◆ Use RNase-free tips and other plastics.

◆ RNA is not contaminated with RNase when in Buffer RL1, but RNase-free plastic and glassware should be used to continue processing after extraction. Glassware can be baked at 150°C for 4 hours, plastic products can be soaked in 0.5M NaOH for 10 minutes, then thoroughly washed with water and autoclaved to remove RNase.

◆ The solution should be prepared using RNase-free water (add water to a treated RNase-free glass bottle, add DEPC to a final concentration of 0.01% (v/v), mix well and leave overnight, autoclaved).

Genomic DNA contamination and removal

The Animal Total RNA Isolation Kit is primarily designed to obtain considerable RNA from animal tissues or cultured cells, and the unique DNA-Cleaning Column effectively removes most of the DNA contamination from the system, and the purified RNA is often available for downstream operations without DNase treatment. Certain RNA analysis experiments are sensitive to trace amounts of DNA, such as fluorescence quantitative RT-PCR analysis of low-abundance genes, where appropriate DNase can be used to further remove DNA contamination.

Procedure (Do Not ice bath or low temperature (4℃) centrifugation)

Before using, add ethanol to Buffer RL2 and Buffer RW2 referring to the label on the bottle.

1. Please lyse cells according to different types and sources.
   a) Adherent cells: Make sure that the cell culture medium has been completely removed, then use Buffer cRL1 (The amount of addition is shown in the table below) to digest and lyse cells. Or add Buffer cRL1 (The amount of addition is shown in the table below) to the collected cells by centrifugation, and then repeatedly pipette the cells (Until aggregated cells cannot be seen).
   Note: please double check that there is no cell culture media left, or it may influence RNA yield and purity.
   b) Suspension cells: collect cells by centrifugation, then add Buffer cRL1 (The amount of addition is shown in the table below), pipette the cells repeatedly (Until aggregated cells cannot be seen).
   Note: The RNA in Buffer cRL1 can resist RNase degradation. The cells can be stored at room temperature for approximately 24 hours if they are not used immediately after being lysed by Buffer RL1. Also, they can be stored at 4℃ for about 1 week, and for longtime storage, please keep them in -80℃. Heat them in 37℃ or leave them at room temperature before use.

Cell culture vessel	Cell amount	Addition amount reagents (μL)	
		Buffer cRL1	Buffer cRL2
96/48/24/12 well plate	< 10^6	250	400
6 well plate/3.5cm plate	~ 10^6	500	800
≥ 6cm plate	>10^6	Suggestion: use Animal Total RNA Isolation Kit (RE-03011), or take no more than 10^6 cells and use Cell Total RNA Isolation Kit (RE-03111)	
Cultured bottle			

2. Transfer the cell lysate to DNA-Cleaning Column (put DNA-Cleaning Column into the collection tube), and centrifuge at 12,000 rpm (~13,400 ×g) for 2 minutes. Remove DNA-Cleaning Column and keep the supernatant in the collection tube.
   Note: Transfer the supernatant to a clean centrifugation tube before step 3, if there is precipitation in the bottom of collection tube.
3. Add Buffer cRL2 into the supernatant (Volume of supernatant: Buffer cRL2 = 1:1.6), mixing gently.
   Note: the addition of the amount of buffer cRL2 is determined by the actual volume of supernatant. For instance, add 400 μL Buffer cRL2 to 250 μL supernatant. If there is flocculation or the mixture solution becomes turbid, please proceed directly to step 4.
4. Transfer the mixture solution (about 700 μL) to RNA-Only Column (put RNA-Only Column into collection tube), centrifuge at 12,000 rpm (~13,400 ×g) for 1 minute, and discard the effluent in collection tube.
   Note: If RNA is isolated from cells in a 6-well plate or 3.5 cm plate, pass the mixed solution through the column in two batches.
5. Put RNA-Only Column back into the collection tube, transfer all the remaining mixture solution to it, centrifuge at 12,000 rpm (~13,400 ×g) for 1 minute, and discard the effluent in collection tube.
6. Add 500 μL Buffer RW1 to RNA-Only Column, centrifuge at 12,000 rpm (~13,400 ×g) for 1 minute, and discard the effluent in collection tube.
7. Add 700 μL Buffer RW2 to RNA-Only Column, centrifuge at 12,000 rpm (~13,400 ×g) for 1 minute, and discard the effluent in collection tube.
8. Repeat step 7.
9. Put RNA-Only Column back into the collection tube, centrifuge at 12,000 rpm (~13,400 ×g) for 2 minutes to remove the remaining Buffer RW2 in RNA-Only Column.

10. Transfer RNA-Only Column to a new RNase-Free centrifugal tube, add 20-50 uL RNase-Free ddH_2O that has already been heated at 65℃ to the center of the membrane of RNA-Only Column. Leave it at room temperature for 2 minutes, centrifuge at 12,000 rpm (~13,400 ×g) for 1 minute, and then collect the RNA solution.
Note: The volume of RNase-Free ddH_2O should not be less than 20 uL, otherwise it may affect the elution efficiency. The RNA solution after centrifugation can be added to the membrane of RNA-Only Column again, and step 10 can be repeated to increase RNA yield.
The product RNA can be used directly in downstream experiment or stored in -80℃. Due to the careful protection of secondary RNA structure supplied by this kit, it is better to leave the product RNA in 72℃ for 5-10 minutes to dissociate the secondary structure before the gel electrophoresis

RNA concentration and purity detection

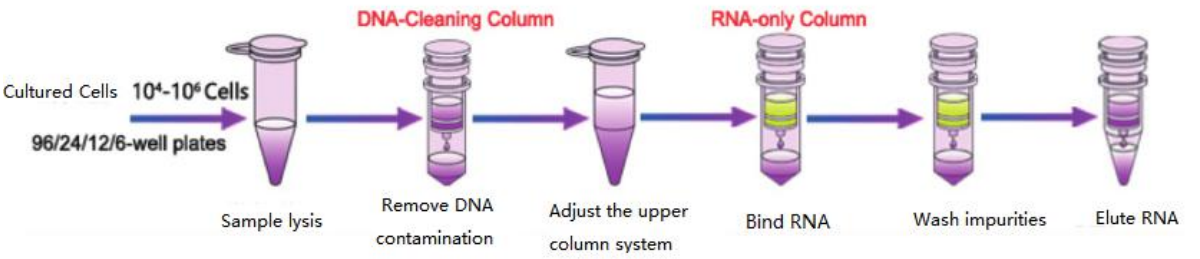
- The quality of the resulting RNA is related to a variety of factors during the procedure. RNA can be measured for concentration and purity using agarose gel electrophoresis and a UV spectrophotometer. Due to the good protection of the secondary structure of RNA in this kit, it is recommended that the resulting RNA solution be denatured at 72°C for 5-10 min before gel electrophoresis.
- RNA concentration is determined with a spectrophotometer with an OD260 value of 1 equivalent to approximately 40 μg/ml of RNA.
- The OD260/280 ratio of RNA is often used as a measure of nucleic acid purity, and in general, the OD260/280 ratio of pure RNA is between 1.8 and 2.1. The OD260/280 ratio is affected by the pH of the solution used for the assay, for example, if the purified RNA reads between 1.9 and 2.1 in 10 mM Tris buffer at pH 7.5, and the ratio decreases to 1.8-2.0 in a neutral aqueous solution, this does not mean that the quality of the RNA changes.

DNA contamination and detection

There is currently no effective purification method to guarantee that the purified RNA is completely free of DNA contamination, and even if DNA bands are not detected during gel electrophoresis, trace amounts of DNA may be present. The RNA extraction kit can remove most of the DNA in the RNA, but trace amounts of DNA may still be present in the sample, and its presence is related to the amount of the sample and its own properties.

Detection of trace amounts of DNA in purified RNA can be performed by real-time PCR detection without reverse transcription. We suggest that primers can be designed with annealed matching regions located in inflets of genomic DNA. If the RNA does not contain any genomic DNA, a PCR based on this primer will not amplify the corresponding PCR product.

Workflow



Problem Analysis Guide

The following analysis of the problems you may encounter in Cell RNA extraction will help you with your experiments. In addition, for other experimental or technical problems in addition to operating instructions and problem analysis, we have dedicated technical support to help you. If you have any needs, please contact us at: 028-83360257 or E-mali : Tech@foregene.com.

RNA is not extracted or RNA yields are low

There are often a variety of factors that affect recovery efficiency, such as: tissue sample RNA content, method of operation, elution volume, etc.

1. Ice bath or cryogenic (4 °C) centrifugation was performed during operation.

   Recommendation: Operate at room temperature (15-25 ° C) throughout the whole process, do not ice bath and centrifuge at low temperatures.

2. Improper sample preservation or excessive sample storage time.

   Recommendation: Store samples at -80 °C or freeze in liquid nitrogen and avoid repeated freeze-thaw use; try to use fresh tissue or cultured cells for RNA extraction.

3. Insufficient sample lysis.

   Recommendation: When homogenizing tissue, ensure that the tissue is sufficiently homogenized and that the tissue cells are sufficiently split to explain the release of RNA.

4. The eluent is not added correctly.

   Recommendation: Confirm that RNase-Free ddH_2O is added dropwise to the middle of the purification column membrane.

5. The correct volume of absolute ethanol was not added to Buffer RL2 or Buffer RW2.

   Recommendation: Follow the instructions, add the correct volume of absolute ethanol to Buffer RL2 and Buffer RW2 and mix well before using the kit.

6. Tissue sample dosage is not appropriate.

   Recommendation: Use 10-20 mg of tissue or (1-5) × 10^6 cells per 500 μl buffer RL1, as excessive tissue use can result in reduced RNA extraction.

7. Improper elution volume or incomplete elution.

   Recommendation: The elution volume of the purification column is 50-200 μl; if the elution effect is not satisfactory, it is recommended to extend the room temperature placement time after adding preheated RNase-Free ddH_2O, e.g. for 5-10 min.

8. The purification column has ethanol residue after Buffer RW2 wash.

   Recommendation: If there is ethanol residue after Buffer RW2 washing, empty tube centrifugation for 1min, the time for the empty tube centrifugation operation can be increased to 2min, or the purification column can be placed at room temperature for 5 min to adequately remove the residual ethanol.

Purified RNA is degraded

The quality of the purified RNA is related to factors such as the preservation of the sample, RNase contamination, and manipulation,etc.

1. Tissue samples are not kept in time.

   Recommendation: If tissue samples or cells are not used in a timely manner after collection, immediately cryopreserve at -80 °C or liquid nitrogen. To extract RNA, use a newly taken tissue or cell sample whenever possible.

2. Repeated freeze-thawing of tissue samples.

   Recommendation: When storing tissue samples, it is best to cut them into small pieces for preservation, and remove one of the pieces when using them to avoid repeated freeze-thawing of the sample and the degradation of RNA.

3. RNase is introduced or not wearing disposable gloves, masks, etc. during the operation.

   Recommendation: RNA extraction experiments are best performed in separate RNA manipulation rooms and the table is cleared before the experiment.

   Wear disposable gloves and masks during the experiment to minimize RNA degradation caused by the introduction of RNase.

4. Reagents are contaminated with RNase during use.

   Recommendation: Replace with a new Animal Total RNA Isolation Kit for related experiments.

5. The centrifuge tubes, tips, etc. used in RNA manipulation are contaminated with RNase.

   Recommendation: Confirm that the centrifuge tubes, tips, pipettes, etc. used in RNA extraction are all RNase-Free.

Purified obtained RNA affects downstream experiments

RNA purified by the purification column, if the salt ions, protein content is too large will affect the downstream experiment, such as: reverse transcription,Northern Blot et al.

1. The elutioned RNA has salt ion residues.

   Recommendation: Confirm that the correct volume of ethanol has been added to Buffer RW2 and perform 2 purification column washes at the centrifugal speed indicated for operation; if there is any salt ion residue, leave the purification column to Buffer RW2 for 5 min at room temperature and perform centrifugation to maximize the removal of salt contamination.

2. Ethanol residue in elutioned RNA.

   Recommendation: Confirm that after buffer RW2 washing, perform the empty tube centrifugation operation at the centrifugation speed indicated for operation, increase the time of the empty tube centrifugation operation to 2 min if there is still ethanol residue, or leave it at room temperature for 5 min after the empty tube centrifugation to maximize the removal of ethanol residue.

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