RNA-easy Isolation Reagent

R701

Vazyme

Instruction for Use

Version 22.1

Contents

01/Product Description

RNA-easy Isolation Reagent is widely applicable to extraction of Total RNA and Small RNA from various animal tissues, plant materials, cultured cells, bacteria and other samples. Compared with the traditional Trizol extraction method, this product is simple to use, eliminating the need to use chloroform for phase separation, and the whole process can be carried out at room temperature. In addition, the product effectively inhibits RNase activity, while simultaneously precipitating impurities such as protein, DNA and polysaccharides to the bottom of the tube, so as to enable single-phase extraction and powerfully guarantee the integrity and purity of the RNA. The whole extraction process can be completed within 50 min with this product. The extracted RNA can be directly used in various molecular biology experiments such as cDNA cloning, qRT-PCR, mRNA purification, *in vitro* translation, Northern blotting hybridization, high-throughput sequencing and so on.

02/Components

Component	R701-01	R701-02
RNA-easy Isolation Reagent	100 ml	200 ml

03/Storage

Store at 2 ~ 8℃. Adjust the shipping method according to the destination.

04/Applications

10 - 50 mg animal tissues, 10 - 100 mg plant tissues, <5 × 10^6 cells, virus sample.

05/Self-Prepared Materials

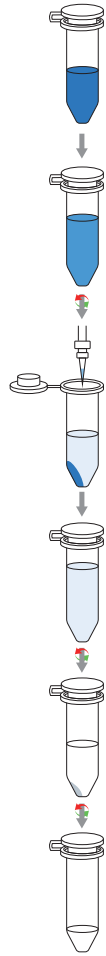
Isopropanol, 75% ethanol (prepared with RNase-free ddH_2O), RNase-free ddH_2O.

06/Notes

1. Protective items such as protective clothing, gloves, goggles and masks, should be worn when using this product as it contains phenol, which is toxic and corrosive. In case of accidental contact with the eyes, immediately wash with plenty of water and go to the hospital for treatment. In case of contact with skin, please wash immediately with a large amount of detergent and water. If you still feel unwell, please go to the hospital for treatment.
2. Preventing RNase contamination is critical for RNA extraction. RNase generally exists in the environment and is extremely stable. A minute amount of RNase can quickly degrade RNA. Therefore, please take protective measures in accordance with the conventional RNA extraction procedure, including wearing masks and clean disposable gloves, working in a separate clean area, using RNase-free laboratory equipment and so on.

07/Mechanism & Workflow

The workflow of RNA-easy Isolation Reagent



Sample lysis

Add RNase-free ddH_2O

Centrifuge at room temperature and precipitate impurities

Transfer supernatant containing the RNA

Add isopropanol

Centrifuge at room temperature

Wash with 75% ethanol

Centrifuge at room temperature

Dissolve the RNA

08/Experiment Process

08-1/Sample Processing

◇ Animal/plant tissue

1. After quick freezing in liquid nitrogen, the fresh tissue is quickly transferred to a mortar pre-cooled with liquid nitrogen and ground with a pestle. Meanwhile, liquid nitrogen is continuously added until the tissue is ground into powder (no obvious particles).
   ▲Incomplete grinding will affect the yield and quality of the RNA.

2. Transfer the pulverized sample to a centrifuge tube, add 500 µl of RNA-easy Isolation Reagent to approximately every 25 mg of tissues and vigorously oscillate or pipette repeatedly to fully lyse the sample.

▲ Every 500 µl of RNA-easy Isolation Reagent can lyse up to 50 mg of conventional tissues. An excessive amount of sample may lead to insufficient lysis and reduce the product purity.

▲ Some tissues with strong tenacity or containing more extracellular matrix may not be lysed completely. The remaining tissue will be precipitated in step 2 of 08-2 and will not affect subsequent RNA extraction and RNA quality.

▲ If liquid nitrogen is not available for grinding, fresh tissue can be cut up as much as possible and soaked in RNA-easy, then homogenized at high speed with an electric homogenizer until the tissue pieces are fully lysed. Alternatively, fresh tissue can be soaked in RNA Keeper Tissue Stabilizer (Vazyme #R501) for effective inactivation of the RNase, then treated with RNA-easy and homogenized with an electric homogenizer until completely lysed.

◇ Suspension cells

1. Collect cells by centrifugation, re-suspend the collected cells in 1 × PBS, centrifuge again and discard the supernatant.
2. Add 500 µl of RNA-easy for every 1 - 5 × 10^6 cells.
3. Pipette repeatedly until the cells are fully lysed.

◇ Adherent cells

1. Discard the cell culture medium and wash once with 1 × PBS.
2. Normally 2 - 3 ml of RNA-easy is added to cells cultured in 10 cm cell culture dish. Add 500 ul of RNA-easy to each well of a conventional six-well plate (well diameter is 3.5 cm) to fully cover the surface of the cells, then pipette the cells.

▲ A cell scraper or clean pipette can be used to remove cells that have adhered firmly to the wall, or trypsin can be used to digest the cells before adding RNA-easy. Subsequently the cells can be processed as suspended cells.

3. Transfer the lysis solution to a centrifuge tube and pipette repeatedly until the cells are fully lysed.

08-2/RNA Extraction

1. Add 2 parts of RNase-free ddH_2O to 5 parts of the above lysis solution (200 µl to 500 µl of RNA-easy), invert to mix and incubate at room temperature for 5 min.
2. Centrifuge at 11,200 rpm (12,000 × g) for 15 min at room temperature.
3. Remove the centrifuge tube in which the solution is now divided into an upper aqueous phase (containing the RNA) and a dark lower precipitate phase (containing impurities such as protein, DNA and polysaccharides). Carefully transfer the upper aqueous phase into a new centrifuge tube.

▲ The volume of the upper aqueous phase accounts for about 90% of the total volume. If we use 500 µl of RNA-easy for extraction and the upper aqueous phase is about 640 µl, it is recommended to transfer 500 µl. All of the supernatant can be transferred in order to reduce RNA loss when extracting from samples containing small amount of RNA.

▲ When the sample input is lower than the recommended amount, there may be no precipitate in the bottom after centrifugation, which is normal. Continue the extraction in accordance to the following steps.

4. Add an equal volume of isopropanol, invert to mix and incubate at room temperature for 10 min.
5. Centrifuge at room temperature at 11,200 rpm (12,000 × g) for 10 min. White precipitate can usually be seen. Carefully discard the supernatant.
   ▲ Some tissues contain a lot of metabolites, resulting in dispersed precipitation. In this case, slowly pipette the supernatant along the liquid surface.
6. Add 500 µl of 75% ethanol (prepared with RNase-free ddH_2O), gently flick the bottom of the tube to suspend the sediment and invert several times.
7. Centrifuge at room temperature at 9,100 rpm (8,000 × g) for 3 min and discard the supernatant.
8. Repeat Step 6 and 7 and discard the supernatant.
   ▲ In order to reduce impure residues, the supernatant should be discarded as much as possible. Centrifuge briefly after discarding most of the supernatant and then remove all the residual liquid.
9. Air-dry at room temperature, add an appropriate amount of RNase-free ddH_2O to dissolve the precipitate, vortex at room temperature for 3 min (or pipette repeatedly) to fully dissolve the RNA precipitate. The extracted RNA products can be stored in aliquots for long term at -85 ~ -65℃ and only be stores for short term at -30 ~ -15℃.
   ▲ Generally, the RNA precipitate can be dried for 2 - 3 min. Do not overly dry. It will be difficult to dissolve the RNA if it is completely dry.
   ▲ The RNA product needs to be fully dissolved, otherwise it will be impossible to accurately quantify the concentration and a low OD_{260}/OD_{280} ratio will be obtained.

08-3/Product Testing

◇ Purity and concentration testing

1. The purity of the product can be determined using a spectrophotometer. OD_{260}/OD_{280} ratio between 1.8 - 2.2 means high RNA purity.
2. The product concentration can be determined using a spectrophotometer. RNA concentration (ng/µl) = OD_{260} × Dilution factor × 40; or directly determined using Qubit.

◇ Integrity testing

Dilute 0.5 - 1 µg of RNA with 1 × TE or RNase-free ddH_2O to 8 µl, then add 1 µl of 10 × DNA loading buffer. Mix thoroughly and load samples to 1% agarose gel for electrophoresis. Or Agilent 2100 Bioanalyzer can be used to determine the RIN of the RNA product.

09/FAQ & Troubleshooting

◇ RNA degradation

RNA degradation may occur in multiple stages. Attention should be paid to the following matters during the experiment:

① Ensure that the reagents and apparatus for extracting RNA are not contaminated by RNase. All centrifuge tubes, pipette tips and related solutions must be free of RNase contamination. High temperature resistant instruments and apparatus can be placed in an oven at 150℃ for 4 - 6 h to eliminate RNase. Other apparatus can be soaked in 0.1% DEPC water overnight;

② Take appropriate protective measures, wear masks and disposable clean gloves and work in a separate clean area;

③ RNA-easy should be added immediately to cells or tissue samples and they should be homogenized rapidly to lyse. If improperly handled, cells or tissue samples will freeze and thaw, RNase will be released to degrade RNA. Tissues with high endogenous RNase content or that are difficult to homogenize should be cut into small pieces, immediately frozen in liquid nitrogen and then ground up. The sample must not thaw during the whole grinding process;

④ The extracted RNA samples need to be properly stored. It is recommended to take a small amount for assay and store the remaining samples in aliquots at -85 ~ -65℃. During electrophoresis, increase the voltage and reduce the gel running time. At the same time, the electrophoresis buffer should be cooled in an ice bath to prevent RNA degradation during electrophoresis.

◇ Presence of contamination

① Protein contamination: If the OD_{260}/OD_{280} ratio of the extracted product is low by spectrophotometer, it indicates that there may be protein contamination. In this case, it is necessary to consider whether too much tissue has been added leading to incomplete lysis. If so, appropriately increase the volume of RNA-easy or reduce the input amount of tissue for extraction;

② Polysaccharide contamination: When testing the extracted product with a spectrophotometer gives a low OD_{260}/OD_{230} ratio, it indicates that polysaccharide contamination may exist. It is necessary to consider whether this was caused by incomplete ethanol removal or an excessive amount of tissue. In this case, it is feasible to appropriately extend the RNA drying time in step 9 of 08-2 or reduce the input amount of tissue for extraction;

③ Fat contamination: When processing fat-rich tissue, the upper layer will be large amount of fat after the step 2 of 08-2 and the clear middle layer can be transferred for the next step.

◇ No precipitate is seen after centrifugation with isopropanol

White precipitate can usually be seen after isopropanol precipitation. If no precipitate is visible, it may be due to low RNA content or a small input amount of tissue. It is recommended to add isopropanol (step 4 of 08-2), place it at 2 ~ 8℃ or -30 ~ -15℃ for 10 - 30 min before centrifuge. When discarding the supernatant in step 5 of 08-2, pipette off the supernatant rather than pouring it off to avoid loss of precipitate. In addition, some tissue materials contain a lot of metabolites resulting in dispersed precipitation. At this point, slowly pipette the supernatant along the liquid surface.

◇ Storage of tissue samples

If RNA can't be extracted immediately, the tissue should be quickly frozen and stored in liquid nitrogen. Alternatively after being quickly frozen in liquid nitrogen, the tissue can be transferred to storage at -85 ~ -65℃. Fresh tissue cannot be directly frozen at -85 ~ -65℃, because the freezing of samples is a slow process during which endogenous RNase will degrade the RNA. In addition, for the situation where there is no liquid nitrogen quick-freezing condition, fresh tissues can be fully soaked in RNA Keeper Tissue Stabilizer (Vazyme #R501) and stored at room temperature for one week, at 2 ~ 8℃ for one month , or long term storage at -30 ~ -15℃ (or -85 ~ -65℃).

◇ What tissue/cell samples can RNA-easy be applied to?

RNA-easy, like traditional Trizol, is a universal Total RNA extraction reagent with a wide range of species and tissues/cells compatibility. RNA-easy has excellent extraction effect on conventional animal tissues (such as liver, heart, kidney, brain, skeletal muscle, etc., excluding spleen and lung), plant tissues (such as rice, corn, arabidopsis thaliana, tobacco, wheat, soybean, etc., excluding cotton) and various cell lines.

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