Ultra-pure Total RNA Extraction Kit Handbook

Samples: animal tissue, cultured cells, blood, plants, etc.

Prepare total RNA in 20-40 min.

Perfect combination of extraction technology and column purification technology.

No need of Chloroform and isopropanol.

Ver：2024/08

Content

Ultra-pure Total RNA Extraction Kit

Composition

Ultra-pure Total RNA Extraction Kit Cat. No.	5 Preps 5003005	50 Preps 5003050
Grinding Rods	5	50
Spin Columns	5	50
Buffer TL	6 ml	55 ml
Buffer EX	1.2 ml	12 ml
Buffer DW (concentrate)	1 ml	10 ml
Buffer WA (concentrate)	1.9 ml	12 ml
Buffer WBR (concentrate)	1.5 ml	10 ml
RNase-free Water	1.5 ml	2 ml×3
Instructions	1	1

Storage

Buffer TL can be transported at room temperature, please store at 2~8℃ after receiving the product. If other items and reagents are stored at room temperature (0~30℃), their performance will remain unchanged within 3 years. If the product is stored at 2~8℃, the validity period can be extended to more than 3 years.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol.
2. 1.5 ml centrifuge tubes (RNase-free 1.5 ml centrifuge tube must be used).
3. Pipettes and tips (to avoid RNase contamination, please use RNase-free pipette tips with filters).
4. Latex gloves, disposable masks and other protective equipment and paper towels.
5. Microcentrifuge (s) (with rotor for 1.5 ml and 2 ml centrifuge tubes).
6. Vortexer.
7. RNase-free RNA extraction laboratory.
8. Liquid nitrogen and mortar, RNA Later (Simgen Cat. No. 4007020), and DNase I on-column digestion kit (Simgen Cat. No. 8010050) may be required.

Quality Assurance

We guarantee that the products provided are passed quality inspection. If the user finds that the product cannot meet the experimental needs during use, please stop using the product immediately and contact Simgen technical support for help. R&D Department, Hangzhou Simgen Biotechnology Co., Ltd. E-mail: technical@simgen.cn, Tel: 400-0099-857.

Description

Buffer TL is a ready-to-use reagent for total RNA extraction from cells and tissues. In homogenized or lysed samples, Buffer TL lyses cells and releases RNA while maintaining RNA integrity. After adding Buffer EX for extraction and centrifugation, the cell lysate is separated into an aqueous phase and an organic phase, and RNA is present in the aqueous phase. Add Buffer DW to the aqueous phase containing RNA, then add the mixture to the Spin Column for centrifugation, the RNA is adsorbed to the Spin Column. After washing with Buffer WA and Buffer WBR to remove residual proteins and PCR inhibitors, elute the RNA with RNase-free Water. The eluted RNA can be immediately used in various molecular biology experiments.

Through the perfect combination of extraction technology and column purification technology, the entire process of RNA extraction with this kit can be completed within 20-40 min. Compared with the Trizol precipitation method, the total RNA obtained by this kit has lower salt and protein residues, and can be directly used for Northern blot analysis, dot hybridization, poly(A)+ selection, in vitro translation, RNase protection analysis and other experiments.

Precautions to prevent RNase contamination

Since RNase activity is stable and resistant to high temperatures, the following precautions must be observed when performing RNA extraction:

1. The laboratory that extracts RNA must be a dedicated separate laboratory, and the RNA-specific laboratory should be as far away as possible from laboratories that frequently use RNase (such as plasmid DNA extraction).
2. Gloves, masks and special lab coats must be worn during the entire RNA extraction experiment. Because saliva and sweat contain RNase, and symbiotic bacteria and molds on the skin are also a source of RNase. Without careful protective measures, these contaminants may remain in the final prepared RNA, causing RNA degradation. Good microbiological handling techniques therefore prevent RNase contamination.
3. Use RNase-free experimental equipment. You must purchase centrifuge tubes and pipette tips marked "RNase-free" (filter tips are recommended) for RNA extraction. If the centrifuge tube or pipette tip does not have an "RNase-free" label, it must be soaked in 0.1% DEPC water at 37℃ overnight and sterilized before use. All equipment and instruments (especially pipettes) used for RNA extraction should NOT be mixed with other laboratories, especially laboratories that frequently use RNase.
4. Freshly collected samples must be used to extract RNA. or freshly collected samples must be stored below -70℃ in a timely manner. Sample pretreatment must be performed at low temperatures, such as grinding samples with liquid nitrogen. Or grinding samples at room temperature with the addition of denaturants (such as Buffer TL, etc.).

Safety Information

Buffer TL contains phenol and guanidinium salts. Contact with skin may cause corrosion. If it accidentally comes into contact with skin, rinse immediately with alkaline detergent and plenty of water. Seek medical help if necessary. Buffer EX and Buffer WA contain irritating compounds. If they accidentally come into contact with your skin or eyes, you must immediately rinse them with plenty of water or saline and seek medical help if necessary.

When working with Buffer TL, Buffer EX, and Buffer WA, please wear latex gloves, masks, protective glasses, and lab coats to avoid contamination of skin, eyes, and inhalation into the mouth and nose.

Applicable Samples

This product can be perfectly used for the extration of total RNA from tissues and cells of various human, animal, plant or bacterial origins. The sample amount are as follows:

Sample	**Amount**
Animal tissues (liver, brain and other easily homogenized tissues)	10~100 mg
Animal tissues (skin, bones, etc.)	100~200 mg
Blood (erythrocytic nucleated organisms)	200 µl
Blood (erythrocytic anucleate)	300~500 µl
Cultured cells	$5\sim10\times10^{6}$
bacteria	50~100 mg
* Plant tissue	50~100 mg

* Some plant tissues have high polysaccharide content are not suitable for this product. It is recommended to use the Polysaccharides & Polyphenolics-rich Plant Total RNA Extraction Kit (Simgen Cat. No. 5103050).

Operation Step Analysis and Explanation

1. Release Sample RNA

(1) For pretreatment of easy-to-treat samples (e.g. cultured cells, blood, etc.), just add 1 ml Buffer TL and pipet with a pipette to release RNA.

(2) For pretreatment of samples that are easy to homogenize (e.g. muscle tissue, liver tissue, etc.), add 500 µl Buffer TL to the 1.5 ml centrifuge tube containing the sample, grind with a Grinding Rod until there is no obvious particle, and then add 500 µl Buffer TL and mix well.

(3) For samples that are difficult to homogenize (e.g. bone tissue, skin tissue, etc.), add liquid nitrogen to the mortar containing the sample to quickly freeze the sample, grind it immediately to powder form, and then add 1 ml Buffer TL to release RNA.

2. Column Purification Technology

(1) RNA binding

Add the same volume of Buffer DW to the aqueous phase containing RNA, then add it to the Spin Column and centrifuge to allow RNA to be adsorbed to the silica gel membrane of the Spin Column. PCR Inhibitors or impurities that inhibit downstream molecular biological reactions are filtered out.

(2) Washing

A. There is usually a small amount of protein remaining on the Spin Column, and Buffer WA can effectively wash away these remaining proteins.

B. Buffer WBR will wash away the Buffer WA remaining on the membrane to ensure that pure RNA is adsorbed on the Spin Column.

C. During the process of RNA binding and washing, only need to filter the solution through the Spin Column. Therefore, there are no strict requirements for centrifugation speed and centrifugation time. You can choose the "short run" mode of the centrifuge to save operating time.

(3) Centrifuge and Spin Dry

Put the washed Spin Column back into the 2 ml Collection Tube, and centrifuge at 14,000 rpm for 1 min (if the centrifuge speed cannot reach 14,000 rpm, we recommend at least centrifuge at 12,000-13,000 rpm for 2 min.) functions:

A. Allow Buffer WBR to be thoroughly centrifuged to remove.

B. During the process of discarding the Buffer WBR filtrate, if any filtrate accidentally contaminates the bottom of the Spin Column, it can also be removed by centrifugation.

(4) Elute RNA

A. We recommend using the RNase-free Water provided in the kit to elute RNA.

B. RNase-free Water incubated to 37℃ can improve the elution efficiency of RNA.

C. After adding RNase-free Water to the Spin Column, extend the incubation time (to 3-5 min) can improve the elution efficiency of RNA.

D. After centrifugation, directly added RNase-free water into the Spin Column to elute RNA. There is no need to open the lid of the Spin Column to evaporate the remaining ethanol. An over-drying Spin Column will cause poor elution of RNA.

E. The eluted RNA fragments range from 200 bp to 10 kb, and the main RNA is ribosomal RNA.

F. For product safety reasons, if the centrifuge does not have a leak-proof lid, we recommend changing the centrifugation to 8000 rpm for 1 min when eluting RNA to prevent the 1.5 ml centrifuge tube lid from falling off.

3. On-Column Digestion of DNA

The kit can remove most of the DNA during the phase separation extraction process. If the purchased cDNA kit already contains a DNase I digestion step (such as cDNA first-strand synthesis kit, Simgen Cat. No. 7306025/7306100), There is no need for a DNase I on-column digestion step. However, if for some sensitive downstream experiments, the remaining trace amounts of DNA must be completely removed, the DNase I on-column digestion kit (Simgen Cat. No. 8010050) can be ordered separately to perform the steps of on-column digestion of DNA, as follows:

Replace step 6 " **Discard the filtrate, put the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA to the Spin Column, centrifuge at 12000 rpm for 30 sec.**"

Replace with the following 3 steps:

(1) Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec. Discard the filtrate and place the Spin Column back into the 2 ml Collection Tube.

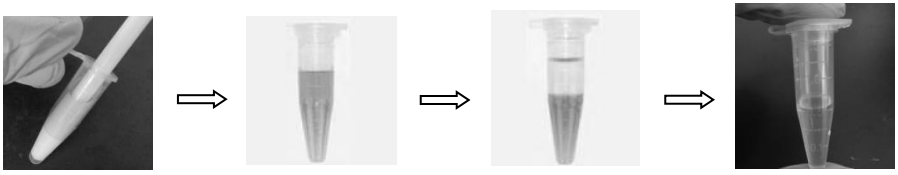
(2) Add 5 µl DNase I to the bottom of an RNase-free 1.5 ml centrifuge tube. Then add 45 µl Buffer RDD. Do not discard the tip. Gently pipette several times to mix evenly. Add the entire mixture to the membrane in the center of the column, incubate at room temperature (20–30℃) for 15 min.

(3) Add 500 µl Buffer WA to the Spin Column, centrifuge at 12,000 rpm for 30 sec.

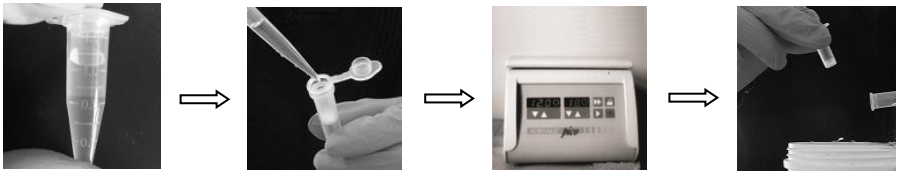
Preparation Before Use

1. If the centrifuge has a refrigeration function, please set the temperature to 25℃.
2. Add absolute ethanol to Buffer DW, Buffer WA and Buffer WBR according to the instructions on the label of the bottle and tick the box on the label to mark "Ethanol Added".
3. Because saliva and skin contain RNase, latex gloves and masks are required throughout the RNA extraction process.

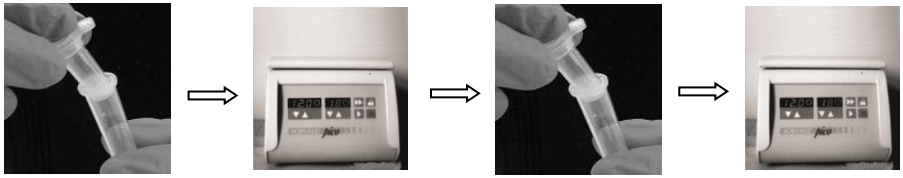
Operation Flow Diagram



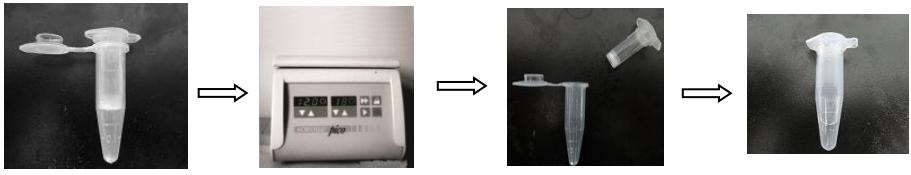
Sample Grinding | Add Buffer TL，Mixing | Add Buffer EX，Mixing，Centrifuge | Take Supernatant



Add Buffer DW | Transfer to Column | Centrifuge bounding RNA | Discarding



Set back column | Washing | Set back column | Spin dry



Put in 1.5 ml tube | Elute RNA | Discard Column | Pure RNA

Protocol for Easily Homogenized Human or Animal Tissues (Muscle, Liver, Brain, Etc.)

1. **Weigh 50~100 mg human or animal tissue, quickly cut into small particles and immediately transfer to a clean 1.5 ml centrifuge tube, add 500 µl Buffer TL, grind with a Grinding Rod until there are no obvious particles, then add 500 µl Buffer TL, shake vigorously for 15 sec to mix.**

* Minimize the time spent to reduce degradation of RNA by endogenous RNase in the tissue.

* Optional step: If the sample contains a lot of protein, fat, polysaccharide or extracellular substances (muscle part, etc.), centrifuge at 12000 rpm for 5 min and take the supernatant. The precipitate obtained by centrifugation includes extracellular membranes, polysaccharides, and high molecular weight DNA, and the supernatant contains RNA. When processing adipose tissue, if there is a large amount of oil on the upper layer, it should be removed, and the supernatant should be taken for the next step.

2. **Add 200 µl Buffer EX, close the lid, shake vigorously for 15 sec, and centrifuge at 12,000 rpm for 15 min.**
3. **Transfer 600 µl supernatant to a clean 1.5 ml centrifuge tube. Add 600 µl Buffer DW. Directly for pipet several times with the tip to mix well and proceed to step 4.**

* Ensure that absolute ethanol has been added to Buffer DW.

4. **Transfer 600 µl mixture to a Spin Column, centrifuge at 12,000 rpm for 30 sec.**
5. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, transfer all the remaining mixture to the Spin Column, centrifuge at 12,000 rpm for 30 sec.**

* The filtrate does not need to be discarded completely. If you want to avoid contamination of the centrifuge by the filtrate adhering to the mouth of the collection tube, you can place the 2 ml Collection Tube upside down on a paper towel and slap it once.

**** If on-column digestion of DNA is required, please refer to "3. On-column Digestion of DNA" on page 5 to replace step 6.***

6. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA to the Spin Column, centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WA.

7. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer WBR to the Spin Column, centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WBR.

8. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge speed cannot reach 14000 rpm, centrifuge at full speed for 2 min.

* Do not omit this step, otherwise the purified nucleic acid may be mixed with ethanol.

9. **Discard the 2 ml Collection Tube, place the Spin Column in an RNase-free 1.5 ml centrifuge tube, add 50 µl RNase-free Water to the Spin Column, incubate at room temperature for 1 min, and centrifuge at 12000 rpm for 30 sec.**

* If the centrifuge does not have a leak-proof lid, please change the centrifugation conditions to 8000 rpm for 1 min to prevent the tube lid from falling off and damaging the centrifuge.

10. **Discard the Spin Column, and the eluted RNA can be used immediately for various molecular biology experiments or stored below -70℃ for later use.**

Protocol for Cultured Animal Cells

For adherent cultured cell samples, please follow step 1a.
For suspension cultured cell samples, please follow step 1b.

1a. Add 1 ml Buffer TL to every 10 cm^2 of cultured cells (for example, in a cell culture dish with a diameter of 3.5 cm, add 1 ml Buffer TL directly after discarding the culture medium). Pipette the cells directly with the pipette tip for several times to lyse the cells, transfer the homogenate to a 1.5 ml centrifuge tube, and proceed to step 2.

1b. Use a 1.5 ml centrifuge tube to collect 5~10×10^6 cells by centrifugation, add 100 µl PBS solution, and vortex until all cells are suspended. Add 1 ml Buffer TL, pipette the cells directly with the pipette tip several times to lyse the cells, and proceed to step 2.

2. **Add 200 µl Buffer EX, close the lid, shake vigorously for 15 sec, and centrifuge at 12,000 rpm for 15 min.**
3. **Transfer 600 µl supernatant to a clean 1.5 ml centrifuge tube. Add 600 µl Buffer DW. Directly pipette several times with the tip to mix well and proceed to step 4.**

* Ensure that absolute ethanol has been added to Buffer DW.

4. **Transfer 600 µl mixture to a Spin Column, centrifuge at 12,000 rpm for 30 sec.**
5. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, transfer all the remaining mixture to the Spin Column, centrifuge at 12,000 rpm for 30 sec.**

* The filtrate does not need to be discarded completely. If you want to avoid contamination of the centrifuge by the filtrate adhering to the mouth of the collection tube, you can place the 2 ml Collection Tube upside down on a paper towel and slap it once.

**** If on-column digestion of DNA is required, please refer to "3. On-column Digestion of DNA" on page 5 to replace step 6.***

6. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA to the Spin Column, centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WA.

7. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer WBR to the Spin Column, centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WBR.

8. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge speed cannot reach 14000 rpm, centrifuge at full speed for 2 min.

* Do not omit this step, otherwise the purified nucleic acid may be mixed with ethanol.

9. **Discard the 2 ml Collection Tube, place the Spin Column into an RNase-free 1.5 ml centrifuge tube, add 50 µl RNase-free Water to the Spin Column, incubate at room temperature for 1 min, centrifuge at 12000 rpm for 30 sec.**

* If the centrifuge does not have a leak-proof lid, please change the centrifugation conditions to 8000 rpm for 1 min to prevent the tube lid from falling off and damaging the centrifuge.

10. **Discard the Spin Column, and the eluted RNA can be used immediately for various molecular biology experiments or stored below -70℃ for later use.**

Protocol for Plant Tissues/Plant Cells/Bacteria

1. **Weigh 300~500 mg sample in a mortar, grind the sample to powder form with liquid nitrogen, and then weigh 50~100 mg of the tissue with a 1.5 ml centrifuge tube pre-cooled with liquid nitrogen. Add 1 ml Buffer TL and shake vigorously for 15 sec to mix.**

* Try to add Buffer TL before the sample is thawed to reduce the degradation of RNA by endogenous RNase in the tissue.

* Optional step: If the sample contains a lot of protein, fat or extracellular substances (plant nodule parts, etc.), centrifuge at 12000 rpm for 5 min and take the supernatant. The precipitate obtained by centrifugation includes extracellular membranes, polysaccharides, and high molecular weight DNA, and the supernatant contains RNA. If the fat content in the sample is high, the upper layer of fat should be removed, and the supernatant should be taken for the next step.

2. **Add 200 µl Buffer EX, close the lid, shake vigorously for 15 sec, and centrifuge at 12,000 rpm for 15 min.**
3. **Transfer 600 µl supernatant to a clean 1.5 ml centrifuge tube. Add 600 µl Buffer DW. Directly pipette several times with the tip to mix well and proceed to step 4.**

* Ensure that absolute ethanol has been added to Buffer DW.

4. **Transfer 600 µl mixture to a Spin Column, centrifuge at 12,000 rpm for 30 sec.**
5. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, transfer all the remaining mixture to the Spin Column, centrifuge at 12,000 rpm for 30 sec.**

* The filtrate does not need to be discarded completely. If you want to avoid contamination of the centrifuge by the filtrate adhering to the mouth of the collection tube, you can place the 2 ml Collection Tube upside down on a paper towel and slap it once.

**** If on-column digestion of DNA is required, please refer to "3. On-column Digestion of DNA" on page 5 to replace step 6.***

6. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA to the Spin Column, centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WA.

7. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer WBR to the Spin Column, centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WBR.

8. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge speed cannot reach 14000 rpm, centrifuge at full speed for 2 min.

* Do not omit this step, otherwise the purified nucleic acid may be mixed with ethanol.

9. **Discard the 2 ml Collection Tube, place the Spin Column into an RNase-free 1.5 ml centrifuge tube, add 50 µl RNase-free Water to the Spin Column, incubate at room temperature for 1 min, centrifuge at 12000 rpm for 30 sec.**

* If the centrifuge does not have a leak-proof lid, please change the centrifugation conditions to 8000 rpm for 1 min to prevent the tube lid from falling off and damaging the centrifuge.

10. **Discard the Spin Column, and the eluted RNA can be used immediately for various molecular biology experiments or stored below -70℃ for later use.**

Protocol for Blood or Bone Marrow

For non-nucleated red blood cell anticoagulated blood or bone marrow samples, please follow step 1a (frozen hemolyzed samples are not applicable).
For nucleated red blood cell anticoagulated blood samples, please follow step 1b.

1a. Add 1.2 ml Red Blood Cell Lysis Buffer to the centrifuge tube, then add 300 µl anticoagulated blood or bone marrow, close the lid and mix evenly by inverting, incubate at room temperature for 5 min, centrifuge at 3000 rpm for 5 min, discard the supernatant, and keep the pellet. Add 1 ml Buffer TL, directly pipette the pellet several times with the tip to lyse the cells and proceed to step 2.

* 300 µl anticoagulated blood can extract 1-2 µg of RNA, if you need to increase the amount of anticoagulated blood, please increase the Red blood cell lysis Buffer in proportion, and keep other conditions unchanged.
* If the anticoagulated blood has been frozen (for example, stored below -70℃), please use the Blood Total RNA Extraction Kit (Simgen Cat. No. 5201050).
* Red Blood Cell Lysis Buffer: NH_4Cl 8.02 g, $NaHCO_3$ 0.84 g, EDTA 0.37 g dissolved in 1 L water. or purchase Red Blood Cell Buffer (Simgen Cat. No.: 9000500) directly.

1b. Add 200 µl blood into a 1.5 ml centrifuge tube, add 1 ml Buffer TL, directly pipette the blood sample several times with the tip to lyse the blood and proceed to step 2.

2. **Add 200 µl Buffer EX, close the lid, shake vigorously for 15 sec, and centrifuge at 12,000 rpm for 15 min.**
3. **Transfer 600 µl supernatant to a clean 1.5 ml centrifuge tube. Add 600 µl Buffer DW. Directly pipette several times with the tip to mix well and proceed to step 4.**

* Ensure that absolute ethanol has been added to Buffer DW.

4. **Transfer 600 µl mixture to a Spin Column, centrifuge at 12,000 rpm for 30 sec.**
5. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, transfer all the remaining mixture to the Spin Column, centrifuge at 12,000 rpm for 30 sec.**

* The filtrate does not need to be discarded completely. If you want to avoid contamination of the centrifuge by the filtrate adhering to the mouth of the collection tube, you can place the 2 ml Collection Tube upside down on a paper towel and slap it once.

**** If on-column digestion of DNA is required , please refer to "3. On-column Digestion of DNA" on page 5 to replace step 6.***

6. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA to the Spin Column, centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WA.

7. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer WBR to the Spin Column, centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WBR.

8. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge speed cannot reach 14000 rpm, centrifuge at full speed for 2 min.

* Do not omit this step, otherwise the purified nucleic acid may be mixed with ethanol.

9. **Discard the 2 ml Collection Tube, place the Spin Column into an RNase-free 1.5 ml centrifuge tube, add 50 µl RNase-free Water to the Spin Column, incubate at room temperature for 1 min, and centrifuge at 12000 rpm for 30 sec.**

* If the centrifuge does not have a leak-proof lid, please change the centrifugation conditions to 8000 rpm for 1 min to prevent the tube lid from falling off and damaging the centrifuge.

10. **Discard the Spin Column, and the eluted RNA can be used immediately for various molecular biology experiments or stored below -70°C for later use.**

Protocol for Human/Animal Bones and Connective Tissues (Skin, etc.)

1. **First, crush the bones with a hammer or chop the tissue. Weigh 400~600 mg sample in a mortar, grind the sample to powder form with liquid nitrogen, Weigh 100~200 mg of powder into a 1.5 ml centrifuge tube pre-cooled with liquid nitrogen, add 1 ml Buffer TL, and shake vigorously for 15 sec to mix.**

* Try to add Buffer TL before the sample is thawed to reduce the degradation of RNA by endogenous RNase in the tissue.

* If the sample amount is small (≤100 mg), you can first add liquid nitrogen to the mortar to grind the sample into powder, then directly add 1 ml Buffer TL to the mortar, and continue grinding until the sample in the mortar was thawed, transfer the homogenate into a 1.5 ml centrifuge tube, and continue with step 2.

2. **Add 200 µl Buffer EX, close the lid, shake vigorously for 15 sec, and centrifuge at 12,000 rpm for 15 min.**
3. **Transfer 600 µl supernatant to a clean 1.5 ml centrifuge tube. Add 600 µl Buffer DW. Directly pipette several times with the tip to mix well and proceed to step 4.**

* Ensure that absolute ethanol has been added to Buffer DW.

4. **Transfer 600 µl mixture to a Spin Column, centrifuge at 12,000 rpm for 30 sec.**
5. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, transfer all the remaining mixture to the Spin Column, centrifuge at 12,000 rpm for 30 sec.**

* The filtrate does not need to be discarded completely. If you want to avoid contamination of the centrifuge by the filtrate adhering to the mouth of the collection tube, you can place the 2 ml Collection Tube upside down on a paper towel and slap it once.

**** If on-column digestion of DNA is required , please refer to "3. On-column Digestion of DNA" on page 5 to replace step 6.***

6. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WA.

7. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer WBR to the Spin Column, centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WBR.

8. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge speed cannot reach 14000 rpm, centrifuge at full speed for 2 min.

* Do not omit this step, otherwise the purified nucleic acid may be mixed with ethanol, which may affect subsequent experimental results.

9. Discard the 2 ml Collection Tube, place the Spin Column into an RNase-free 1.5 ml centrifuge tube, add 50 µl RNase-free Water to the Spin Column, incubate at room temperature for 1 min, and centrifuge at 12000 rpm for 30 sec.

* If the centrifuge does not have a leak-proof lid, please change the centrifugation conditions to 8000 rpm for 1 min to prevent the tube lid from falling off and damaging the centrifuge.

10. Discard the Spin Column, and the eluted RNA can be used immediately for various molecular biology experiments or stored below -70℃ for later use.

Troubleshooting Guide

1. Spin Column clogged

(1) The RNA content in the sample is too high. Please refer to " Applicable Samples" on page 3 to reduce the amount of sample used.

(2) Polysaccharide in the sample is too high. For example, some plant tubers, fruits, seeds and aging leaves (mainly polysaccharide derivatives of starch) and cartilage tissue (cartilage is a polysaccharide substance), etc. If the Spin Column is blocked, please stop using the product immediately and contact our dealer for return or exchange.

* Most samples that have problems due to high polysaccharide content can be solved by changing the kit to the Plant Total RNA Extraction Kit (Simgen Cat. No. 5101050). If it is a sarcocarp sample (with high moisture content), it is recommended to choose the Sarcocarp Total RNA Extraction Kit (Simgen Cat. No. 5102050). Some plant samples or fungal samples that produce special mucopolysaccharides may clog the Spin Column when extracting RNA with the Plant Total RNA Extraction Kit, so you must choose the Polysaccharides & Polyphenolics-rich Plant Total RNA Extraction Kit (Simgen Cat. No. 5103050) to extract RNA.

2. RNA Degradation

(1) Fresh tissue: Some samples rich in endogenous nucleases (such as liver, thymus, etc.) cannot be homogenized directly. The tissue should be ground into a homogenate with a Grinding Rod while adding part of Buffer TL.

(2) Sample storage: Tissue samples should be quickly frozen in liquid nitrogen immediately after collection, and then moved to a -70℃ refrigerator for storage. cell samples should be added to 1 ml Buffer TL directly after collection, and then moved to a -70℃ refrigerator for

storage. If it cannot be stored in a -70℃ refrigerator immediately, RNA Later (Simgen Cat. No. 4007020/4007100) can be purchased. When using RNA Later, the following should be noted:

A. Only use fresh samples that have not been repeatedly frozen and thawed and add RNA Later for storage.

B. Do not store the sample at room temperature for a long time after adding RNA Later. The RNA Later has a time limit for storing samples. Generally, it can only be stored for 1 day at 37℃. It can be extended to 1 week at 15-20℃, and to 4 weeks at 2-8℃. If stored for a long time, should be stored below -20℃.

(3) Contamination of exogenous RNase: RNase in reagents, equipment and experimental environment enters the experimental system. Refer to "Precautions to Prevent RNase Contamination" on page 2 to improve experimental conditions and environment and ensure that RNA is extracted in a dedicated laboratory for RNA extraction.

(4) For electrophoresis detection, it is recommended to use formaldehyde denaturing gel electrophoresis (refer to page 540 of the third edition of Molecular Cloning).

3. Low RNA Extraction Yield

(1) The RNA content in the sample is low. Please refer to " Applicable Sample" on page 3 to appropriately increase the amount of sample used.

(2) The samples were high in polysaccharides. For example, in some plant tissues and cartilage tissues (cartilage is a polysaccharide material), the RNA yield may be very low. The reason is that polysaccharides will form an insoluble precipitate with Buffer TL, and RNA will be wrapped in the precipitate. If you encounter the above situation, please stop using the product immediately and contact our dealer for return or exchange.

(3) The sample is not sufficiently broken or homogenized incompletely. Please refer to " Applicable samples" on page 3 to select the appropriate sample starting volume and increase the homogenization time.

(4) Absolute ethanol is not added to Buffer DW, Buffer WA, or Buffer WBR. Please ensure that absolute ethanol has been added to Buffer DW, Buffer WA or Buffer WBR before using the kit.

(5) The elution efficiency is low. Please refer to point 4 "Elute RNA " in " Column Purification Technology " on page 4 to optimize the RNA elution scheme.

4. Subsequent RNA experiments performed poorly

(1) Too much salt remains. Pay attention to the washing order of Buffer WA and Buffer WBR to ensure that the Spin Column is washed in the correct order.

(2) Too much ethanol remains. Note that the high-speed spin step cannot be omitted, and the Spin Column after separation should be carefully removed and avoided inversion to prevent the residual filtrate at the bottom of the 2 ml Collection Tube from contacting the Spin Column.

(3) Too much RNA was used as a reverse transcription template. It is usually more appropriate to add 100~1000 ng RNA as a template in a 20 μl reverse transcription reaction system. Note that cDNA needs to be appropriately diluted when used as a PCR template to prevent residual reverse transcriptase (including inactivated reverse transcriptase) from interfering with the activity of Taq polymerase.

(4) Effect of reverse transcribed DNA-RNA complex on fluorescent PCR. It is recommended to reduce the random primers or use specific primers for reverse transcription or add RNase H after reverse transcription to remove DNA-RNA complexes.

5. DNA residue

(1) Too many samples are used. Please refer to "Applicable samples " on page 3 to select the appropriate sample starting amount.

(2) When absorbing the aqueous phase, the intermediate phase was absorbed. Carefully and slowly absorb the aqueous phase. If the intermediate phase is accidentally absorbed, pour the aqueous phase back into the centrifuge tube, close the lid, shake vigorously for 15 sec, centrifuge at 12,000 rpm for 15 min, replace with a new tip and carefully absorb the aqueous phase.

(3) The phases are not completely separated. After adding Buffer EX, make sure to shake vigorously to mix, and centrifuge at 12,000 rpm for 15 min. Do not use vortexing instead of shaking.

(4) Samples were previously treated with other reagents. Make sure the starting sample does not contain organic solvents (e.g., ethanol, DMSO), or alkaline reagents that can interfere with phase separation.