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Universal Total RNA Extraction Kit

Composition

Universal Total RNA Extraction Kit Cat. No.	5 Preps 5010005	50 Preps 5010050
Spin Columns	5	50
2 ml Collection Tubes	5	50
β-mercaptoethanol	50 µl	500 µl
Buffer RLA	4 ml	40 ml
Buffer RLK	3 ml	30 ml
Buffer WBR (concentrate)	2 ml	20 ml
Buffer RDD	250 µl	2.5 ml
DNase I	28 µl	270 µl
RNase-free Water	1.5 ml	2 ml×3
Instructions	1	1

Storage

DNase I can store at -20℃, other reagents can store at room temperature (0~30℃) for up to 2 years without showing any reduction in performance and would be stable more than 2 years if stored at 2-8℃ (the product stored at 2~8℃ should be restored to room temperature before use).

Introduction

This product provides customers with a set of high quality, complete total RNA extraction from animal tissues, plant tissues, cultured cells, bacteria in a small number of solutions. The kit uses a unique cell cracking system, without the use of phenol, chloroform, and other harmful substances, through the spin column silicon matrix membrane efficiently and specifically adsorbed nucleic acid molecules, and then through DNase treatment to remove genomic DNA pollution, and finally obtain high purity total RNA.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol.
2. RNase-free 1.5 ml centrifuge tubes.
3. Pipette and tips (RNase-free pipette tips with filter element is recommended to avoid RNase contamination).
4. Disposable gloves, protective equipment, and paper towels.
5. Microcentrifuge(s) (with rotor for 1.5 ml and 2 ml centrifuge tubes).
6. Water bath and vortexer.
7. A laboratory for RNase use.
8. DEPC may be required to treat water (for bacterial samples).

Preparation before use

1. Add β-mercaptoethanol to the Buffer RLA at 1% (V/V) (for example, add 10 µl β-mercaptoethanol to 1 ml Buffer RLA), cap the prepared lysate tightly and mark the bottle. The Buffer RLA added with β-mercaptoethanol can be stored at -20℃ for up to one month.
2. Add absolute ethanol to the Buffer WBR according to the instructions on the label of the reagent bottle and tick the box on the label to mark "Ethanol added".
3. Because RNase are present in saliva and skin, wear latex gloves and a mask during the entire RNA extraction process.

Protocol

Before the experiment, please refer to the table listed in the most suitable starting dosage of various samples, to prepare the sample lysate.

Table 1. The optimal dosage of different samples, Buffer RLA and Buffer RLK

Sample Name	Sample Dosage	Buffer RLA Dosage	Buffer RLK Dosage
Common animal tissues (liver, kidney, heart, lungs, brain, etc.)	5-20 mg	300 µl	300 µl
Common animal tissue (liver, kidney, heart, lungs, brain, etc.)	20-40 mg	500 µl	500 µl
Special animal tissue (spleen, etc.)	5- 10 mg	300 µl	300 µl
Suspended/adherent cells.	1.5×10^3-5×10^6	300 µl	300 µl
Plant tissue (leaves, stems)	30-50 mg	300 µl	300 µl
Plant tissue (leaves, stems)	50- 100 mg	500 µl	500 µl
Bacteria (when OD600 reaches 0.6- 1.0)	0.5 ml	200 µl	300 µl

* Note: In the preparation of sample lysate, if the lysate prepared is very viscous (such as: spleen, cells) and not easy to transfer, you can use a 20G syringe to suck back and forth several times, to facilitate the absorption of lysate.

* The content of polysaccharide polyphenol in some plant tissues is high, which is not suitable for this product. It is recommended to use the Polysaccharides & Polyphenolics-rich Plant Total RNA Kit (Simgen Cat. No.5103050).

* The prepared lysate can be stored at -70℃ for later use.

1. Treatment of samples from different sources

(1) Preparation of animal tissue lysates:

According to the following two methods A and B for the preparation of lysate:

A. Fresh or frozen tissue must be used. Quickly place the tissue into an RNase-free 1.5 ml centrifuge tube or homogenate tube, add Buffer RLA (as recommended in Table 1), place in the ice, and break up the cells with a tissue homogenizer.

B. Fresh or frozen tissue must be used. Quickly place the tissue into a mortar that has been added with liquid nitrogen for grinding, constantly replenishing the liquid nitrogen during the grinding process to prevent the tissue from thawing, completely ground the tissue to a powder form. Quickly transfer the tissue to the RNase-free 1.5 ml centrifuge tube for weighing. When the remaining liquid nitrogen was about to evaporate, add Buffer RLA (according to the recommended dosage in Table 1) and pipette several times until there was no obvious bulk tissue in the lysate.

(2) Preparation of plant tissue lysate:

Fresh or frozen tissue must be used. Quickly place the tissue into a mortar that has been added with liquid nitrogen for grinding, constantly replenishing the liquid nitrogen during the grinding process to prevent the tissue from thawing, completely ground the tissue to a powder form. Quickly transfer the tissue to the RNase-free 1.5 ml centrifuge tube for weighing. When the remaining liquid nitrogen was about to evaporate, add Buffer RLA (according to the recommended dosage in Table 1) and pipette several times until there was no obvious bulk tissue in the lysate.

* It is recommended to choose younger tissues to extract RNA. Mature or senescent plant tissues have low RNA content and more starch and other polysaccharide derivatives, which may lead to jelly-like lyse products, resulting in failure of RNA extraction. If the lyse products are jelly-like, it is recommended to select the Polysaccharides & Polyphenolics-rich Plant Total RNA Kit (Simgen Cat. No.5103050).

(3) Preparation of cell lysate:

① Adherent cell lysate can be prepared by the following two methods A and B:

A. **Trypsin treatment method**

a. Pour away the culture solution, wash the cells with sterile 1×PBS solution, and then add trypsin solution just enough to cover the monolayer cells: for example, add 2 ml trypsin solution in a 150 mm culture bottle, add 1 ml trypsin solution in a 100 mm culture dish, gently shake the dish to evenly distribute trypsin on the cell layer until the cells become loose (usually 1-2 min).

b. Once the cells are loose, remove the trypsin solution as soon as possible (tilt the plate or culture bottle to remove excess solution with the tip), add 1×PBS solution, and blow the attached cells.

c. Transfer the cells to an RNase-free 1.5 ml centrifuge tube and centrifuged 300-500×g (~1100-1500 rpm) for 5 min, discard the supernatant.

d. Add Buffer RLA (as recommended in Table 1), pipette to mix well. Transfer the lysate to an RNase-free 1.5 ml centrifuge tube.

B. **Scraping separation method:**

a. Discard the medium, add appropriate amount sterile 1×PBS, pipette the cells until the cells detach from the dish or flask. For firmly attached cultured cells, the cells can be peeled off with a cell scraper.

b. Wash the cells with PBS, centrifuged at 300-500×g (~1100- 1500 rpm) for 5 min, discard the supernatant to collect cells.

c. Add Buffer RLA (as recommended in Table 1), pipette to mix well, transfer the lysate to an RNase-free 1.5 ml centrifuge tube.

② **Preparation of suspended cell lysate:**

A. Transfer the suspended cells together with the culture medium to an RNase-free 1.5 ml centrifuge tube and centrifuged at 300-500×g (~1100- 1500 rpm) for 5 min. Discard the supernatant to collect cells.

B. Add Buffer RLA (as recommended in Table 1), pipette to mix well, transfer the lysate to an RNase-free 1.5 ml centrifuge tube.

(4) Preparation of bacteria lysate:

A. Culture the bacteria to OD_{600} 0.6-1.0, add 0.5 ml bacterial culture solution to a 1.5 ml centrifuge tube, and centrifuge at 12000 rpm for 30 sec.

B. Discard the supernatant to collect the bacteria.

* Some divalent cations will inhibit lysozyme activity, if the bacterial medium contains divalent cations (such as MRS Medium, etc.), a washing step should be added after centrifugation to collect the bacteria: Add 1 ml distilled water, vortex to suspend the bacteria, centrifuge at 12000 rpm for 30 sec, discard the supernatant.

C. Add 50 µl DEPC water for suspension pellet, then add 50 µl 3 mg/ml lysozyme solution prepared by DEPC water, vortex to mix well, incubate at 37℃ for 5-10 min.

* The thinner cell walls of Gram-negative bacteria can reduce the concentration of lysozyme solution to 0.4 mg/ml.

D. Add Buffer RLA (as recommended in Table 1), pipette to mix well, transfer the lysate to an RNase-free 1.5 ml centrifuge tube.

2. Add Buffer RLK (as recommended in Table 1), vortex to mix well, incubate at room temperature for 3-5 min.

* If the source of the sample (animal tissue and cells) is precious, it can be incubated at 70℃ for 3 min after adding Buffer RLK to improve the yield of RNA.

* If there are still lumpy pellet after vortex, pipette several times until the pellet disappear.

3. Animal tissue lysate, plant tissue lysate and cell lysate should be centrifuge at 12000 rpm for 5 min after mix. Transfer the supernatant carefully to an RNase-Free 1.5 ml centrifuge tube. The bacteria lysate can be directly proceed to the next step without centrifugation.

* After centrifugation, if a layer of solid substance is formed on the surface of the supernatant, just move the solid substance to the side of the centrifuge tube with the tip before transfer the supernatant.

4. Add 0.5 times the supernatant volume of absolute ethanol, vortex to mix well, so that the liquid becomes cloudy and foamy.

* If there is blocky precipitation after vortex, pipette several times until the precipitation disappears. The absolute ethanol must be thoroughly mixed with the supernatant. If the mixing is uneven, the final RNA yield will be affected.

5. Transferred 750 µl mixture to a Spin Column (the Spin Column was placed in a 2 ml Collection Tube). Close the lid and centrifuged at 12000 rpm for 1 min.

6. Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, transfer the remaining mixture to the Spin Column, close the lid and centrifuge at 12000 rpm for 1 min.

* The filtrate does not need to be completely abandoned. To avoid contamination of the centrifuge by the filtrate adhering to the mouth of the collection tube, you can slap the 2 ml Collection Tube upside down on a paper towel twice.

7. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube. Add 600 µl Buffer WBR, close the lid, centrifuge at 12000 rpm for 30 sec, and discard the filtrate.**

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

8. **Mix 45 µl Buffer RDD with 5 µl DNase I to make incubate solution.**

* Gently pipette to mix well, do not vortex.

* The incubation solution configured in this step is the amount of extracting one tube of RNA, do NOT store the incubation solution.

9. **Add 50 µl incubation solution to the Spin Column center and incubate at room temperature for 15 min.**
10. **Add 800 µl Buffer WBR, close the lid and centrifuge at 12000 rpm for 30 sec.**
11. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 300 µl Buffer WBR, close the lid and centrifuge at full speed (≥13000 rpm) for 1 min.**
12. **Discard the 2 ml Collection Tube and place the Spin Column in a clean RNase-free 1.5 ml centrifuge tube. Add 50~100 µl RNase-free Water to the Spin Column center, close the lid, incubate at room temperature for 2 min and centrifuged at 12000 rpm for 1 min.**

* When taking out the Spin Column, do not let the filtrate touch the bottom of the Spin Column. If the Spin Column is contaminated with filtrate, please discard the filtrate, and place the Spin Column back into the 2 ml Collection Tube centrifuge at the full speed for 1 min, and then takeout the Spin Column to perform this step.

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

Table 2. Reference yield of RNA extracted from different materials.

Type of sample	Sample name	RNA yield	A260/A230	A260/A280
Animal tissue	Mouse liver	3.0-5.5 µg/mg	2.0-2.5	1.9-2.1
	Mouse kidneys	1.5-3.0 µg/mg	2.0-2.5	1.9-2.1
	Mouse spleen	1.5-3.5 µg/mg	2.1-2.6	1.9-2.1
	Mouse heart	0.4- 1.0 µg/mg	2.0-2.6	1.9-2.1
	Mouse lung	0.6- 1.2 µg/mg	2.2-3.0	1.9-2.1
	Mouse brain	0.4-0.8 µg/mg	2.1-2.7	1.9-2.1
Plant tissue	Tomato leaves	1.0-2.0 µg/mg	2.1-2.6	1.9-2.2
Cells	293T (1×10^6)	8- 10 µg/ time	2.0-2.3	1.9-2.1
	Hela cell (1×10^6)	20 µg/ time	2.0-2.3	1.9-2.1
Bacteria	*E.Coli* (1×10^9)	30-50 µg/ time	2.2-2.6	1.9-2.1