TIANGEN®

RNA Easy Fast Tissue/Cell Kit

For purification of total RNA from animal tissue and cultured animal cell

www.tiangen.com/en

RNA Easy Fast Tissue/Cell Kit

(Spin Column)

Cat.no. 4992732

Kit Contents

Contents	4992732 (50 preps)
Buffer RLA	30 ml
Buffer RW3	40 ml
Buffer RW	12 ml
Proteinase K	500 µl
RNase-Free ddH_2O	15 ml
gDNA Eraser Columns set	50
RNase-Free Columns CR4 set	50
RNase-Free Centrifuge Tubes (1.5 ml)	50
Handbook	1

Optional Reagents

DNase I (1500 U) (TIANGEN, Cat. no. 4992232)

Storage Conditions

Store the kit at 15-30°C for 15 months.

For the optional reagent RNase-Free DNase I, store at 2-8°C for 15 months.

Introduction

The RNA Easy Fast Tissue/Cell Kit is a rapid RNA extraction kit for animal tissues/cells developed based on the genomic DNA removal technology exclusively developed by TIANGEN. This kit does not contain toxic reagents such as β -mercaptoethanol or DTT, and a large number of different samples can be processed at the same time with the single RNA extraction be completed within 30 minutes. The total RNA extracted from this product has high yield, good purity, an no protein or other impurities pollution. The purified RNA can be used in RT-PCR, real time RT-PCR, chip analysis, Northern Blot, Dot Blot, PolyA screening, *in vitro* translation, RNase protection analysis, molecular cloning and other downstream experiments.

Notes of preventing RNase contamination

1. Change gloves frequently, because the skin contains bacteria, which may cause RNase contamination.
2. Use RNase-Free plastic products and tips to avoid cross-contamination.
3. RNA will not be degraded by RNase in Buffer RLA. However, in the following procedures after extraction, RNase-Free plastic and glassware should be used. To remove RNase, heat the glassware at 150°C for 4 hours, or soak the plastic ware in 0.5 M NaOH for 10 minutes, then washed thoroughly with water, and send for sterilizing.

Precautions

1. If the subsequent experiments have strict requirements for RNA purity, DNase I can be selectively added for digestion. Please refer to optional DNase I digestion step in the protocol. DNase I is not supplied in this kit, please refer to the optional reagents for the cat. no. 4992232 for purchasing.
2. Before the first use, absolute ethanol should be added into Buffer RW. Please refer to the label on the bottle for the ethanol volume.
3. If not specified, the following operations shall be carried out at room temperature.

Reagents need to be prepared by yourself

96-100% ethanol and 70% ethanol

Protocol

Please add 96-100% ethanol into Buffer RW according to the instructions on the label.

I Extraction of total RNA from animal tissue

1. Sample pretreatment
   Add 350 µl of Buffer RLA to every 10-20 mg of tissue. Homogenize the tissue thoroughly with an electric homogenizer, then add 10 µl of Proteinase K to the sample. After homogenization, incubate at room temperature for 5 min.
   Note: It is recommended to use 5 mg of spleen tissue and 50-100 mg of muscle tissue.
2. Centrifugation at 12,000 rpm (~ 13,400 × g) for 2-5 min, save the supernatant and carry out the following operations.
3. Transfer the supernatant to the gDNA Eraser Column set, centrifuge at 12,000 rpm (~ 13,400 × g) for 30 sec, and collect the flow-through.
4. Slowly add 70% ethanol with the same volume of the supernatant to the above filtrate, mix well (precipitation may occur at this time), and transfer the obtained solution and precipitation together into the RNase-Free Column CR4 Set (the adsorption column is placed in the collection tube). Centrifuge at 12,000 rpm (~ 13,400 × g) for 30 sec, discard the waste liquid, and then place the RNase-Free Column CR4 back in the collection tube.
5. If DNase I digestion is not needed, add 700 µl of Buffer RW3 to the RNase-Free Column CR4, centrifuge at 12,000 rpm (~ 13,400 × g) for 30 sec, discard the waste liquid, and put the RNase-Free Column CR4 back in the collection tube.
6. DNase I digestion (optional): if the requirements for RNA purity are strict in subsequent experiments, DNase I digestion can be carried out selectively.
   1) Add 350 µl of Buffer RW3 to the RNase-Free Column CR4, centrifuge at 12,000 rpm (~ 13,400 × g) for 30 sec, discard the waste liquid, and put the RNase-Free Column CR4 back in the collection tube.
   2) Preparation of DNase I reaction solution:
      Dissolve DNase I dry powder (1500 U) in 550 µl RNase-Free ddH_2O, mix gently, and store at -30~-15°C after aliquoting (can be stored for 9 months).
      Note: The DNase I storage solution thawed from -30~-15°C shall be kept at 2-8°C (can be kept for 6 weeks), and do not freeze it again.
   3) Transfer 10 µl of DNase I storage solution to a new RNase-Free centrifuge tube, add 70 µl of Buffer RDD and mix gently to make the DNase I working solution.
   4) Add 80 µl of DNase I working solution to the center of the RNase-Free Column CR4, and place it at room temperature for 15 min.
   5) Add 350 µl of Buffer RW3 to the RNase-Free Column CR4, centrifuge at 12,000 rpm (~ 13,400 × g) for 30 sec, discard the waste liquid, and put the RNase-Free Column CR4 back in the collection tube.

7. Add 500 µl of Buffer RW **(Ensure that ethanol (96-100%) has been added to Buffer RW before use)** to the RNase-Free Column CR4, leave it at room temperature for 2 min, centrifuge at 12,000 rpm (~ 13,400 × g) for 30-60 sec, discard the waste liquid, and put the RNase-Free Column CR4 back in the collection tube.
8. Repeat step 7.
9. Centrifuge at 12,000 rpm (~ 13,400 × g) for 2 min, and then discard the waste liquid. Place the RNase-Free Column CR4 at room temperature for 2 min to completely dry the residual washing buffer in the column.
   Note: The purpose of this step is to remove the residual washing buffer in the RNase-Free Column CR4. The residual washing buffer may affect the subsequent RT experiments.
10. Put the RNase-Free Column CR4 into a new RNase-Free centrifuge tube, add 30-100 µl of RNase-Free ddH_2O to the center of the adsorption membrane, place it at room temperature for 2 min, and centrifuge at 12,000 rpm (~ 13,400 × g) for 2 min to obtain RNA solution.
    Note: The volume of the elution buffer should not be less than 30 µl, since too small volume will affect the recovery efficiency. Please store the RNA solution at - 70°C.

II Extraction of total RNA from cultured cells

1. Collecting cells:
   a. Collection of suspended cells (the number of cells to be collected should not exceed 1×10^7): Estimate the appropriate number of cells, centrifuge at 300 × g for 5 min, collect the cells into a centrifuge tube, and carefully remove all the supernatant of the medium.
   b. Collection of monolayer adherent cells (the number of cells to be collected should not exceed 1×10^7): The cells can be directly lysed in a culture dish (the diameter of the dish should not exceed 10 cm), or be collected by centrifugation after treatment with trypsin. (the monolayer adherent cells cultured in flask are usually treated with trypsin.)
      1) Lyse directly in the cultured dish: First determine the number of cells, centrifuge to collect the cell pellet, then completely remove the supernatant of cell culture medium, and immediately carry on to the second step of lysis.

2) Trypsin treatment: First determine the number of cells, remove the culture medium, wash the cells with PBS. Remove PBS, then add PBS containing 0.10-0.25% trypsin to the cells, when the cells detach from the container wall, add the culture medium containing serum to inactivate trypsin. Transfer the cell solution to a RNase-Free centrifuge tube, centrifuge at 300 × g for 5 minutes to collect the cell pellet, and carefully remove all supernatant.

Note: When collecting cells, it is necessary to completely remove the cell culture medium, otherwise it will lead to incomplete lysis, and affect the binding of RNA to the adsorption column, thus reducing the RNA yield.

2. Cell lysis

For the collected cell pellet: Gently flick the bottom of the tube to loosen the cell pellet, then add proper amount of Buffer RLA and 10 µl of Proteinase K to resuspend the cells. Vortex to mix.

Cell number	Buffer RLA (µl)
$<5\times10^6$	350
5×10^6-1×10^7	600

For directly lysed cells: Refer to the following table for the usage of Buffer RLA. Transfer the cell lysate to the centrifuge tube, vortex to mix.

Diameter of the dish (cm)	Buffer RLA (µl)
<6	350
6-10	600

3. Centrifuge at 12,000 rpm (~ 13,400 × g) for 2-5 min, save the supernatant and carry out the following operations.
4. Transfer the supernatant to the gDNA Eraser Column set, centrifuge at 12,000 rpm (~ 13,400 × g) for 30 sec, and collect the flow-through.
5. Slowly add 70% ethanol with the same volume of the supernatant to the above filtrate, mix well (precipitation may occur at this time), and transfer the obtained solution and precipitation together into the RNase-Free Column CR4 set (the adsorption column is placed in the collection tube). Centrifuge at 12,000 rpm (~ 13,400 × g) for 30 sec, discard the waste liquid, and then place the RNase-Free Column CR4 back in the collection tube.
6. If DNase I digestion is not needed, add 700 µl of Buffer RW3 to the RNase-Free Column CR4, centrifuge at 12,000 rpm (~ 13,400 × g) for 30 sec, discard the waste liquid, and put the RNase-Free Column CR4 back in the collection tube.

7. DNase I digestion (optional): If the requirements for RNA purity are strict in subsequent experiments, DNase I digestion can be carried out selectively.
   1) Add 350 µl of Buffer RW3 to the RNase-Free Column CR4, centrifuge at 12,000 rpm (~ 13,400 × g) for 30 sec, discard the waste liquid, and put the RNase-Free Column CR4 back in the collection tube.
   2) Preparation of DNase I reaction solution:
      Dissolve DNase I dry powder (1500 U) in 550 µl RNase-Free ddH_2O, mix gently, and store at -30~-15°C after aliquoting (can be stored for 9 months).
      Note: The DNase I storage solution thawed from -30~-15°C shall be kept in 2-8°C (can be kept for 6 weeks), and do not freeze it again.
   3) Transfer 10 µl of DNase I storage solution to a new RNase-Free centrifuge tube, add 70 µl of Buffer RDD and mix gently to make the DNase I working solution.
   4) Add 80 µl of DNase I working solution to the center of the RNase-Free Column CR4, and place it at room temperature for 15 min.
   5) Add 350 µl of Buffer RW3 to the RNase-Free Column CR4, centrifugate at 12,000 rpm (~ 13,400 × g) for 30 sec, discard the waste liquid, and put the RNase-Free Column CR4 back in the collection tube.
8. Add 500 µl of Buffer RW **(Ensure that ethanol (96-100%) has been added to Buffer RW before use)** to the RNase-Free Column CR4, leave it at room temperature for 2 min, centrifuge at 12,000 rpm (~ 13,400 × g) for 30-60 sec, discard the waste liquid, and put the RNase-Free Column CR4 back in the collection tube.
9. Repeat step 8.
10. Centrifuge at 12,000 rpm (~ 13,400 × g) for 2 min, and then discard the waste liquid. Place the RNase-Free Column CR4 at room temperature for 2 min to completely dry the residual washing buffer in the column.
    Note: The purpose of this step is to remove the residual washing buffer in the RNase-Free Column CR4. The residual washing buffer may affect the subsequent RT experiments.
11. Transfer the RNase-Free Column CR4 into a new RNase-Free centrifuge tube, add 30-100 µl of RNase-Free ddH_2O to the center of the adsorption membrane, place it at room temperature for 2 min, and centrifuge at 12,000 rpm (~ 13,400 × g) for 2 min to obtain RNA solution.
    Note: The volume of the elution buffer should not be less than 30 µl, since too small volume will affect the recovery efficiency. Please store the RNA solution at - 70°C.