ELK Biotechnology

For research use only.

RNA clean Kit

Catalog No.	Specification	Storage/Shelf life
EP014-50T	50T	Room temperature/1 year

Introduction

The reagents used in this kit do not contain phenol and chloroform, which greatly reduces the harm of phenol and chloroform to the experimenter and expands the use environment. This product can quickly extract total RNA from animal cells, tissues and blood, and can process a large number of different samples simultaneously. Extracted total RNA with high purity and extremely low contamination of proteins and other impurities, which can be used for RT-PCR, Real Time RT-PCR, chip analysis, Northern Blot, Dot Blot, PolyA screening, in vitro translation, RNase protection analysis and molecular cloning Various downstream experiments.

Kit Components

Component	EP014-50T
Lysate REL	**15 ml**
RNA deproteinized Buffer RRPB	**60 ml**
Washing Buffer RWB	**60 ml**
Proteinase K	**1 vial (stored at -20 ° C)**
DNase I stock solution	**1 vial (stored at -20 ° C)**
DNase I Buffer RDB	**4ml**
R column	**50sets**
RNase-Free ddH2O	**40ml**
User Manual	**1 copy**

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I. Preparation before use

RWB: Please add absolute ethanol to RWB (labeled on the reagent bottle) before use.

Operation steps

1. Sample processing:

For adherent cell samples

Carefully aspirate the medium with a pipette, add 300 μL of **RNA Extraction Lysate Buffer REL**, and repeatedly blow the cells into the lysate with a cell scraper or pipette, and transfer the mixture to a 1.5 mL EP tube;

For suspended cell samples

Centrifuge at 300 × g for 5 min at 4 ° C to collect the cell pellet in a 1.5 mL EP tube, discard the supernatant, add 300 μL of lysate **REL**, and repeatedly mix by pipetting;

For animal and plant tissue samples

Take 20mg of tissue in 300μL **REL** and fully grind (use glass homogenizer or electric homogenizer, provided by the customer), carefully transfer the mixed solution into 1.5mL EP tube (excessive amount of tissue affects

lysate efficiency, The proportion of lysate can be increased in proportion);

2. Add 590 μL of RNase-Free ddH2O and 10 μL of **Proteinase K** to the homogenized sample, mix thoroughly with shaking, and bath in 56 ° C for 10 min.
3. Centrifuge at 12,000 rpm (~ 13,400 × g) for 5 min. Take the supernatant in a new 1.5mL EP tube and perform the following operations.
4. Slowly add 0.5 times the volume of supernatant ethanol (prepared by the customer), mix upside down (do not shake vigorously, precipitation may occur at this time), and transfer the obtained solution and precipitation to the R column (adsorption The column was placed in a collection tube), centrifuged at 10,000 rpm (~ 10,000 × g) for 1 min, the waste liquid in the collection tube was discarded, and the adsorption column R column was returned to the collection tube. (The adsorption column can be filled with 750 μl of solution at one time. If the solution and precipitation cannot be added all at once, please transfer to the R column of the adsorption column several times).

5. Add 500 μl of deproteinized solution **RRPB** to the R column of the adsorption column and centrifuge at 10,000 rpm (~ 10,000 × g) for 1 min.
6. Preparation of **DNase I** working solution: Take 10 μl of DNase I stock solution in a new RNase-Free EP tube, add 70 μl of DNase I buffer RDB, and mix (DNase I working solution is better to be prepared when use).
7. Add 80 μl of DNase I working solution to the R column of the adsorption column and leave it at room temperature for 10 min.
8. Add 500 μl of deproteinized solution **RRPB** to the R column of the adsorption column, and centrifuge at 10,000 rpm (~ 10,000 × g) for 1 min.
9. Add 500 μl of **RWB** washing solution to the R column of the adsorption column (please check whether anhydrous ethanol has been added before use), let it stand at room temperature for 2 minutes, and centrifuge at 12,000 rpm (~ 13,400 × g) for 1 minute. Return the R column to the collection tube.
10. Repeat step 9.
11. Centrifuge at 12,000 rpm (~ 13,400 × g) for 2 minutes and discard the waste liquid. Place the adsorption column R column in a clean

bench for several minutes to completely dry the remaining rinsing solution in the adsorption material.

Note: The purpose of this step is to remove the remaining rinsing solution from the R column of the adsorption column. The remaining rinsing solution may affect subsequent RT and other experiments.

12. Transfer the R column of the adsorption column to a new RNase-Free centrifuge tube, and add 30-100 μl of RNase-Free ddH2O to the middle of the adsorption membrane. Drop at room temperature for 2 min, 12,000 rpm (~ 13,400 × g) Centrifuge for 2 min to obtain RNA solution.

Note: The volume of elution buffer should not be less than 30 μl. Too small volume will affect the recovery efficiency. Store RNA solution at -70 ° C.

Detection of RNA purity and concentration

Integrity: RNA can be detected by ordinary agarose gel electrophoresis (electrophoresis conditions: gel concentration 1.2%; 1 × TAE running buffer; 120V, 20 min). Since 70% -80% of the RNA in the cells is rRNA, you should see very obvious rRNA bands under UV after electrophoresis. The amount of 28S rRNA is about twice that of 18S rRNA, indicating that the integrity of the RNA is better.

Purity: The OD260 / OD280 ratio is an indicator of the degree of protein contamination. High-quality RNA, OD260 / OD280 readings are between 1.8-2.1, and a ratio of 2.0 is a hallmark of high-quality RNA. The OD260 / OD280 reading is affected by the pH of the solution used in the assay. The same RNA sample, assuming an OD260 / OD280 reading of 1.8-2.1 measured in a 10 mM Tris, pH 7.5 solution, may read between 1.5-1.9 in an aqueous solution, but this does not indicate RNA Impure.

Concentration: Take a certain amount of RNA extract, dilute n times with RNase-Free ddH2O, zero the spectrophotometer with RNase-Free ddH2O, take the diluted solution for OD260 / OD280 measurement, and calculate the RNA concentration according to the following formula:

Final concentration (ng / μl) = (OD260) × (dilution multiple n) × 40