Total RNA Extraction Kit

#Cat: NB-66-32297-50T Size : 50T
#Cat: NB-66-32297-200T Size : 200T

Catalog No.	Specification	Storage/Shelf life
NB-66-32297-50T	50T	Room temperature/1year
NB-66-32297-200T	200T	Room temperature/1year

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 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, invitro translation, RNase protection analysis and molecular cloning Various downstream experiments.

Kit components

Component	NB-66-32297-50T	NB-66-32297-200T	Storage
Lysate REL	15 ml	60 ml	RT
RNA deproteinized Buffer RRPB	70 ml	140 ml*2	RT
Washing Buffer RWB	60 ml	240 ml	RT
Proteinase K	525 µl	2.1 ml	-20℃
DNase Ⅰ stock solution	525 µl	2.1 ml	-20℃
DNase Ⅰ Buffer RDB	5ml	20ml	RT
Adsorption column R column	50 sets	200 sets	RT
RNase- Free ddH_2O	40ml	160ml	RT
User Manual	1 copy	1 copy	RT

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**, Pipette the lysate up and down several times 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 300µ L of lysate **REL**, and repeatedly mix by pipetting;

For animal and plant tissue samples

Take 20mg of tissue in 300 µL lysate **REL** and fully grind (use glass homogenizer or electric homogenizer, self-provide), 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 ddH_2O 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 new1.5mL EP tube and perform the following operations.

4. Slowly add 0.5 times the volume of supernatant ethanol (self-prepared), 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, discard the waste liquid in the collection tube, and return the adsorption column R column to the collection tube. (The adsorption column can be filled with 750µl 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 750 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. （**Choose to do**）Preparation of **DNase** working solution: Take 10 µl of DNase Ⅰ stock solution in a new RNase-Free EP tube, add 90 µl of DNase Ⅰ buffer RDB, and mix (DNase Ⅰ working solution is better to be prepared right before use).

7. （**Choose to do**）Add 100 µl of DNase I working solution to the R column of the adsorption column and leave it at room temperature for 10 min.

(Steps 6 and 7 are the steps to remove DNA, whether it is necessary to choose according to the needs of subsequent experiments)

8. （Choose to do） add 600 µl of deproteinized solution **RRPB** to the R column of the adsorption column, stand for 2 min, and centrifuge at 10,000 rpm (~ 10,000 ×g) for 1min. (If the protein content of the sample is high, which can choose to add the step of removing protein)
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 ddH_2O to the middle of the adsorption membrane. Stand 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). Since70%- 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 OD_{260} / OD_{280} ratio is an indicator of the degree of protein contamination. High-quality RNA, OD_{260} / OD_{280} readings are between 1.8-2.1, and a ratio of 2.0 is a hallmark of high-quality RNA. The OD_{260} / OD_{280} reading is affected by the pH of the solution used in the assay. The same RNA sample, assuming an OD_{260} / OD_{280} reading of 1.8-2.1 measured in a 10 mM Tris, pH 7.5 solution, may read between 1.5-1.9inan aqueous solution, but this does not indicate RNA Impure.

Concentration:

Take a certain amount of RNA extract, dilute n times with RNase- FreeddH_2O, zero the spectrophotometer with RNase-Free ddH_2O, take the diluted solution for OD_{260} / OD_{280} measurement, and calculate the RNA concentration according to the following formula: Final concentration (ng /µl) = (OD_{260}) × (dilution multiple n) ×40.

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