For research purpose only. Not for use in diagnostic procedures for clinical purposes. For IN VITRO USE ONLY.

easy-spin™ (DNA free) Total RNA Extraction Kit

Cat. No. **17221** 50 Rxn

DESCRIPTION

There are largely two types of products used in extracting RNA, which are column type and solution type.
The advantages of solution type (phenol use) products are that their yield is higher than that of spin type ones and they can extract a relatively larger quantity of RNA. Although they are inconvenient in that they use phenol and have to go through the process of alcohol PPT(precipitation), they are economically efficient in extracting RNA. For these reasons, they are preferred by many customers.
On the contrary, spin type products do not use phenol and do not have to go through the process of alcohol PPT, so they can extract purer RNA. Regardless they are relatively expensive, they are prefered for convenience and promptness when RNA should be extracted from many samples or quickly.
RNA extracted using the two types of products is pure enough to be used in almost all kinds of molecular biology experiments including Northern blot analysis, cDNA synthesis and RT-PCR.
However, when electrophoresis is performed on RNA extracted using products above, genomic DNA on the top is occasionally contaminated. This occurs mainly because the optimal cell number suggested by the manufacturers is not observed and, as a result, the overload genomic DNA is not removed sufficiently in the lysis stage. Customers may experience that many times.
Even if genomic DNA is contaminated, it is not a big problem in general experiments but the contamination of genomic DNA can have significant negative influences on important experiments.
In order to avoid this problem, the present company developed a RNA extracting kit without genomic DNA contamination. easy-spin™ (DNA free) Total RNA Extraction Kit combines the advantages of solution type products and column type ones, removing the inconveniency of alcohol PPT process in solution type products and enabling the extraction of total RNA within 30 minutes without genomic DNA. The most remarkable characteristics of the easy-spin™ Kit is: (1) there is no genomic DNA contamination, (2) there is no alcohol PPT process, and (3) as a result RNA extraction time is less then 30 minutes.

STORAGE

Lysis Buffer : Store at 4°C, after receiving.
Other components : Store at Room temperature

KIT CONTENTS

• Lysis Buffer	50ml
• Binding Buffer	20ml
• Washing Buffer A	40ml
• Washing Buffer B	10ml
: Before use, add 40ml of absolute ethanol(EtOH)	
• Elution Buffer	20ml
• Columns	50 Columns
• Collection Tubes	50 Tubes

PREPARING SOLUTION BEFORE USE

- Chloroform
- 100% EtOH

HOMOGENIZATION TECHNIQUES

1. *For Tissues* : Homogenize tissue samples in 1ml of Lysis Buffer (easy-BLUE™ reagent) per 50-100mg of tissue using a Homogenizer or equivalent. The sample volume should not exceed 10% of the volume of Lysis Buffer used for homogenization.
2. *For Cells (grown in monolayer)* : Lyse cells directly in a culture flask by adding 1ml of Lysis Buffer(easy-BLUE™ reagent) per 3.5cm diameter. An insufficient amount of reagent may result in contamination of the extracted total RNA with DNA and protein.
3. *For Cells (grown in suspension)* : Pellet cells by centrifugation. Lyse cells in this reagent by repetitive pipetting. Wahing cells before addition of Lysis Buffer(easy-BLUE™ reagent) should be avoided, because this increases the possibility of RNA degradation. Disruption of some yeast and bacterial cells may require the use of a homogenizer.

PROTOCOL (For Cell)

1. Prepare 1-10x10^6 cell in 1.5ml tube. Centrifuge it to remove culture media (13,000rpm, 10sec), and add 1ml of Lysis buffer(easy-BLUE™ reagent).
   Note : In case of adherent cell, measure the viable count after trypsin treatment. In case of suspended cell, measure the viable count after centrifugation. Although 1ml of Lysis Buffer is good for the preparation of up to 5-10x10^6 cell, it is recommended not to exceed 3-10x10^6 cell because RNA purity may fall with higher cell counts. Besides, in case of adherent cell, we can treat Lysis Buffer in culture flask after removing culture medium, but doing so would waste a large amount of reagents and may result in the loss of harvested cell lysate. In any case, it is recommended to use after treatment of trypsin. Generally speaking, a T75 flask filled with adherent cells to about 75-80% volume would have 7-8X10^6 cell. In such case where an exact cell count is difficult to measure, use about 1/3 of volume and come up with an approximated cell count. However, it is always better to keep accurate cell count.
2. Vigorously vortex in room temperature for 10sec.
   Note : This is actual cell lysis stage and is thus important to apply vortex until no clumps are seen. Once the cell is lysed, store it at 4℃. The sample is now stable at 4℃ up to a week.
3. Add 200μl of Chloroform and apply vortex.
   Note: Observe the tube before vortexing. When Chloroform is added, one would see a white line being formed just beneath the upper (blue layer)
   as the chloroform layer moves down. This region contains mixed parts of cell debris, protein, and genomic DNA and RNA. The purpose of adding the chloroform is to separate the phenol layer from aqueous layer and to eventually isolate RNA and genomic DNA/protein.
4. After centrifuging the solution at 13,000 rpm (4℃) for 10 min, transfer 400μl of the upper fluid to an empty 1.5ml tube.
   Note : Centrifugation of the solution creates two phases. The upper aqueous phase contains RNA while the lower phenol layer(blue color) contains denatured protein or cell debris. White sediments are visible between two phases. This interface contains mixtures of protein and genomic DNA. Protein and genomic DNA can be isolated from this interface (Methods available upon request). When pipetting the upper layer, pay attention to to form any white sediments.
5. Add 400μl of Binding Buffer and mix it well by pipetting or gently inverting the 2-3 times. Do not centrifuge and leave it for 1min at room temperature.
6. Load the upper solution to the column, but do not load the whole upper solution because the maximum volume of the column reservoirs is 800μl. After loading the optimum of the upper solution to the column, and centrifuge at 13,000rpm for 30sec. Discard the flow-through after centrifuging and place the spin column back in the same 2ml collection tube. And then repeat this step.
   Note : The maximum volume of the column reservoirs is 800μl. For same volume or larger volume, reload the remained sample in the column and spin again.
7. Add 700μl of Washing buffer A to the column. Close the tubes gently, and centrifuge for 30 sec at 13,000rpm to wash the column. Discard the flow-through and place the spin column back in the same 2ml collection tube.
8. Wash by adding 700μl of Washing buffer B to the column and centrifuge for 30 sec at 13,000rpm. Discard the filtrates and place the spin column back in the same 2ml collection tube.
   Note : Washing buffer B is supplied as a concentrate. Ensure that ethanol is added to Washing buffer B before use.
9. Centrifuge for 1-2 min at 13,000rpm to dry the column membrane.
   Note : It is important to dry the column membrane since residual ethanol may interfere the downstream reactions.
10. Place the column in a clean 1.5ml microcentrifuge tube (not provided), and add 50μl of Elution buffer directly onto the membrane. Incubate at RT for 1min, and centrifuge for 1min at 13,000rpm to elute.

PROTOCOL (For Tissue)

1. Preparation of 50-100mg of fresh tissue.
2. Add 1ml of Lysis Buffer(easy-BLUE™ reagent) and homogenize tissue sample using a homogenizer or equivalent.
   Note : Homogenize tissue samples in 1ml of Lysis Buffer per 50-100mg of tissue using a homogenizer or equivalent. The sample volume should not exceed 10% of the volume of Lysis Buffer used for homogenization.
3. For preparation of RNA from tissue, follow step 2 of protocol (for cells).