easy-spin™ [DNA free] Total RNA Extraction Kit

The Instruction Manual for Total RNA Extraction from tissues and cells.

RUO *Research Use Only* REF *17221* Σ 50 RT

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.

KIT CONTENTS

Label	Description	Contain
Lysis Buffer (easy-BLUE™ Solution) [1]	Lysis reagent	50 ml
Binding Buffer	Binding Solution	20 ml
Washing Buffer A	Washing Buffer	40 ml
Washing Buffer B [2] : Before use, add 40 ml of absolute Ethanol(EtOH)	Washing Buffer	10 ml
Elution Buffer	Elution Buffer	20 ml
Spin Column	Nucleic acid binding column	50 Col.
Collection tube	2 ml polypropylene tube	50 tubes
Instruction Manual	-	1 ea

[1] **Lysis Buffer : Store at 4ºC, after receiving.**
Other components : Store at Room temperature

[2] **Washing Buffer B is supplied as a concentrate. Ensure that the 40 ml of ethanol is added to Washing Buffer B before use.**

STORAGE AND STABILITY

- Lysis Buffer : Store at Store at 4 ºC, after receiving.
- Other components : Store at room temperature.
- Stable for 2 years.

APPLICATIONS

- Express messenger RNA research
- Detection Assay : RT-PCR, real time PCR
- Pathogen detection study
- RNA hybridization : Northern blotting, microarray

PRODUCT USE LIMITATIONS

easy-spin™ (DNA free) Total RNA Extraction Kits are developed, designed, and sold for research purpose only. They are not to be used for human or animal diagnostic or therapeutic uses. Do not use internally or externally in humans or animals. Be careful in the handling of the products.

PRODUCT WARRANTY AND SATISFACTION GUARANTEE

All products undergo extensive quality control test and are warranted to perform as described when used correctly. Immediately any problems should be reported. Satisfaction guarantee is conditional upon the customer providing full details of the problem to iNtRON within 60 days, and returning the product to iNtRON for examination.

PREPARING SOLUTION BEFORE USE

- Common equipment and reagents
  - Equipment for disruption and homogenization, including Grinding Jar Set (mortar)
  - Pipettes and pipette tips
  - Vortex mixer
  - 2-propanol
  - Microcentrifuge tubes (1.5 ml)
  - Liquid nitrogen
  - Other general lab equipments
  - Water bath or heating block
  - Chloroform or bromochloropropane
  - Microcentrifuge with rotor for 2.0 ml tubes
  - Ice
  - DEPC-treated water or RNase-free water

HOMOGENIZATION TECHNIQUES

- *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.
- *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.
- *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 50-100 mg 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).

EXPERIMENT INFORMATIONS

❖ Total RNA preparation with several companies's products

easy-spin™ (DNA free) Total RNA Extraction Kit is provides a simple and rapid method for the isolation of total RNA from cultured cells and tissues

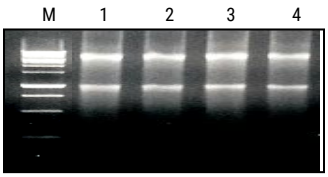

Fig. 1. Gel Analysis of Total RNA isolated from several companies's products
Total RNA was purified from several companies's product using the solution and spin type products. And then total RNA was analyzed in gel electrophoresis. 2μl of eluted solution was loaded per lane on a 1.0% agarose gel.
Lane M, 1Kb ladder Maker; **Lane 1,** easy-BLUE™ Kit; **lane 2,** easy-spin™ Kit; **lane 3,** Supplier A(solution type); **lane 4,** Supplier B(spin type)

❖ Effectively result of removing gDNA using the easy-spin™ Kit

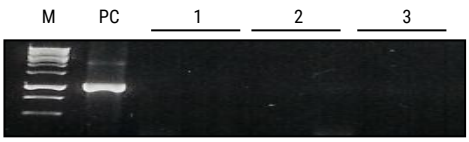

Fig. 3. PCR Amplification for IL-10 gene with *i*-StarTaq™ DNA Polymerase of iNtRON
Total RNA was purified from cell using easy-BLUE™ Kit, RNA-spin™ Kit and easy-spin™ Kit. And then, RNA from easy-BLUE™ Kit and RNA-spin™ Kit were treated DNase after purifying RNA. RNA from easy-spin™ Kit was not treated DNase. And then PCR reaction was performed using *i*-StarTaq™ DNA Polymerase. It was analyzed in gel electrophoresis. 5μl of eluted solution was loaded per lane on a 1.0% agarose gel.
Lane M, marker DNA; **lane 1,** PCR of RNA from easy-BLUE™ Kit after treating DNase; **lane 2,** PCR of RNA from RNA-spin™ Kit after treating DNase; **lane 3,** PCR of RNA from easy-spin™ (DNA free) Kit

❖ RT-PCR Result

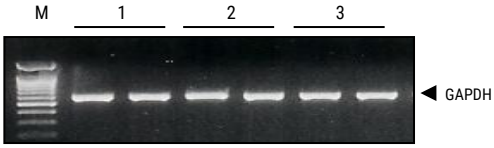

Fig. 4. RT-PCR Amplification for GAPDH gene with ONE-STEP RT-PCR PreMix Kit of iNtRON
Total RNA was purified from cell using several RNA extraction kit of different products. And then, the first strand cDNA was synthesized and its PCR reaction using ONE-STEP RT-PCR PreMix Kit. It was analyzed in gel electrophoresis. 5μl of eluted solution was loaded per lane on a 1.0% agarose gel.
Lane M, marker DNA; **lane 1,** PCR of RNA from easy-BLUE™ Kit; **lane 2,** : PCR of RNA from RNA-spin™ Kit; **lane 3,** PCR of RNA from easy-spin™ (DNA free) Kit

❖ Yield and purity of iNtRON's related products

Product Name	Yield	Purity
easy-BLUE™ Kit	17~22㎍	1.87 ~ 2.02
RNA-spin™ Kit	14~18㎍	1.95 ~ 2.05
easy-spin™ Kit	15~20㎍	1.96 ~ 2.09

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommendation
Low RNA yield or no RNA	Too much starting material	- Do not overload the sample, overloading significantly reduces yield. Reduced the amount of starting material
	Sample integrity is poor	- Samples that were not homogenized or frozen immediately upon isolation may have decreased amount of RNA with reduced integrity. Freeze tissue immediately in liquid nitrogen and store at –70°C if they cannot be immediately processed. Homogenized samples should be stored at -20°C or -70°C.
	Insufficient homogenization	- Homogenize until visible tissue fragment are eliminated.
	Step were not followed correctly or wrong reagent used	- Check the protocol; Washing buffer B did not contain 100% EtOH so, 100% EtOH must be added to the Washing buffer B before use.
	Lysate allowed to overheating during homogenization	- If overheating is a problem, lysate can be placed on ice. Work as quickly as possible.
	Incomplete removal of supernatant	- Check the step 1 of protocol I; When processing cultured cells ensure complete removal of the supernatant after cell harvesting.
RNA degradation	RNA degraded during sample preparation	- It is essential to work quickly during sample preparation. - RNA in sample material is subject to degradation by intracellular RNases until it is frash frozen and homogenized in the presence of RNase-inhibiting or denaturing agents. Therefore, it is imperative that samples are immediately flash frozen in liquid nitrogen and stored at –70°C or are processed as soon as harvested.
	Inappropriately handled	- Use DEPC-treated glassware and wear gloves at all time.
RNA does not perform well in the downstream application	Ethanol carryover	- Ensure that during the Washing buffer B, the easy-spin™ column is spun at maximum speed 1min to dry the easy-spin™ column

RELATED PRODUCTS

Product	Cat. No.
R&A-BLUE™ Total RNA Extraction Kit	17501
easy-RED™ Total RNA Extraction Kit	17063
easy-RED™ BYF Total RNA Extraction Kit	17064
Maxime™ RT PreMix (Oligo $(dT)_{15}$ Primer)	25081
Maxime™ RT PreMix (Random Primer)	25082
Maxime™ RT-PCR PreMix	25131
ONE-STEP RT-PCR PreMix Kit	25101
RNase WiPER	21131
RedSafe™ Nucleic Acid Staining Solution	21141
Agarose LE	32034