ISO 9001/14001 Certified Company

RNA-spin™ Total RNA Extraction Kit [for Cell/Tissue]

Cat. No. 17211 50 Columns

DESCRIPTION

RNA-spin™ Total RNA Extraction Kit is designed for rapid isolation of total RNA from cells, tissues. The purified RNA is ready for use such as cDNA synthesis, RT-PCR, Northern blot, dot blot, primer extension, et al… RNA-spin™ Kit uses advanced silica-gel membrane technology for rapid and efficient purification of RNA without organic extraction or ethanol precipitation. Furthermore, the chaotropic salt in lysis buffer inactivates immediately RNase to ensure isolation of intact RNA. So, RNA-spin™ buffer system is optimized to allow rapid and simple cell lysis followed by selective binding of RNA to the column. Thus the purification procedure is less time consuming compared with alternative methods which require extraction with organic solution, RNA precipitation or ultracentrifugation. RNA-spin™ procedure is very simple, so you can purify RNA from a variety of target source in less than 30 min.

STORAGE

Store at room temperature.

KIT CONTENTS

- R-buffer 20ml
  : Before use, must be add 10μl β-mercaptoethanol per 1ml R-buffer.
- Washing buffer A 40ml
- Washing buffer B 10ml
  : Washing buffer B is supplied as a concentrate. Before using for the first time, add 40ml of absolute EtOH.
- Elution buffer 20ml
- Columns 50 columns
  : Silica membrane based, polypropylene tube
- Collection tube 50 tubes
  : polypropylene tube for 2ml

NOTES FOR BEFORE USING RNA-SPIN™ KIT

☐ RNases can be introduced accidentally into the RNA extraction through improper technique. Because RNase activity is difficult to inhibit, it is Essential to prevent in advance. Always wear disposable gloves. Also, use sterile, disposable plasticware and automatic pipettes reserved for RNA work to prevent cross-contamination with RNase from shared equipment.

☐ DNase digestion is not required since the RNA-spin silica-membrane technology efficiently removes most of the DNA without DNase treatment. However, further DNA removal may be necessary for certain RNA applications. In these cases, the small residual amounts of DNA remaining can be removed using RNase-free DNase by a DNase digestion after RNA purification.

PROTOCOL I (For total RNA from Animal Cells)

1. Harvest cells (do not use more than $5x10^6$ cells) in 1.5ml tube. Centrifuge it to remove culture media (13,000rpm, 10sec).
   Note : In case of adherent cells, measure the viable count after trypsin-EDTA treatment. In case of suspended cell, measure the viable count after centrifugation. Incomplete removal of the cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for binding of RNA to the RNA-spin column membrane. Therefore after centrifugation, remove the remnant with a pipette. Besides, depending on yield and purity, it may be possible to increase the cell number in subsequent preparations. But do not overload the column. Overloading will significantly reduce yield and purity.
2. Add 350μl of freshly prepared R-buffer and vigorously vortex at room temperature for 30 sec. Then, incubate the lysate at room temperature for 5-10 min and one more vigorously vortex at room temperature for 30 sec.
   Note : For pelleted cells, loosen the cell pellet thoroughly by repetitive tapping the tube before use, Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced yields. Ensure that β-mercaptoethanol is added to R-buffer. The 350μl of R-buffer is good for the preparation of up to $5x10^6$ cell. If more than $5x10^6$-$1x10^7$ cells are processed, add more R-buffer. Do not use more than $1x10^7$ cells.
3. Add 350μl (1 volume of R-buffer) of 70% EtOH (not provided) to the lysate and mix well by pipetting or gently invert. Do not centrifuge.
4. Load cell lysates to the column and centrifuge at 13,000rpm for 30 sec. Discard the flow-through after centrifuging and place the spin column back in the same 2ml collection tube.
   Note : The maximum volume of the column reservoirs is 800μl. For larger volume, sample reload and spin again.
5. Add 700μl of Washing buffer A to the RNA-spin™ 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.
6. 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 before use.
7. Centrifuge for 2 min at 13,000rpm to dry the RNA-spin™ membrane.
   Note : It is important to dry the RNA-spin membrane since residual ethanol may interfere with downstream reactions.
8. 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 II (For Cytoplasmic RNA from Animal Cells)

1. Harvest the cells as described in Protocol I.
2. Add 175μl of cold Cytoplasmic lysis buffer (not provided) to lysis the plasma membrane, and incubate on ice for 5min.
   Note : Before adding Cytoplasmic lysis buffer, precool to 4°C.
   [**Cytoplasmic lysis buffer** (50mM Tris-Cl, pH8.0; 140mM NaCl; 1.5mM $MgCl_2$; 0.5% (v/v) Nonidet P-40)]
3. Centrifuge lysate at 4°C for 2min at 13,000rpm. Transfer supernatant to a new 1.5ml tube (not provided), and discard the pellet.
   Note : The supernatant is the cytoplasmic extract and the pellet contains nuclei and cell debris.
4. Add 600μl of prepared R-buffer to the supernatant and vigorously vortex.
   Note : Before use, must be add 10μl β-mercaptoethanol per 1ml R-buffer.
7. Add 430μl of absolute EtOH (not provided) to the lysate and mix well by pipetting or gently invert. Do not centrifuge.
8. Proceed to step 5 in Protocol I.

PROTOCOL III (For total RNA from Animal Tissues)

1. Prepare freshly 10-20mg of tissue. Do not use more than 30mg.
   Note: It is essential to begin with the correct amount of tissue in order to obtain optimal RNA yield and purity with RNA-spin™ column.
2. Disrupt tissue and homogenize lysate in prepared R-buffer.
   Note : Before use, must be add 10μl of β-mercaptoethanol per 1ml R-buffer. The 350μl of R-buffer is good for the preparation of up to 20mg but add more R-buffer if it is difficult to lysis.
3. Vigorously vortex at room temperature for 30 sec. Then, incubate the lysate at room temperature for 5-10 min and one more vigorously vortex at room temperature for 30 sec.
4. Centrifuge the tissue lysates for 3 min at maximum speed at 4°C, and carefully transfer the supernatant to a new 1.5ml tube (not provided) by pipetting.
5. Proceed to step 3 in Protocol I.

TECHNICAL INFORMATION

EXPERIMENTAL INFORMATION

• *Total RNA preparation from different cells and tissue*

RNA-spin™ Total RNA Extraction Kit for Cell/Tissue is provides a simple and rapid method for the isolation of total RNA from cultured cells, tissues and bacteria.

1> Cells

Species and Material	Amount (Cells)	Total RNA Yield (㎍)	RNA Purity
SNU 5 (Human)	1 x 10^6	12-18 ㎍	2.02
K562 (Human)	1 x 10^6	10-20 ㎍	2.0
B16 (Mouse)	1 x 10^6	17-25 ㎍	2.0
Vero cell (Monkey)	1 x 10^6	12-20 ㎍	2.04
E.coil	5x 10^6	55-58 ㎍	2.01

2> Tissues

Species and Material	Amount (Tissue)	Total RNA Yield (㎍)	RNA Purity
Spleen (Mouse)	10mg	20-32 ㎍	2.01
Kidney (Mouse)	10mg	22-33 ㎍	2.0
Liver (Mouse)	10mg	38-40 ㎍	2.0

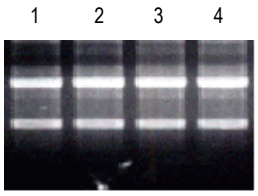

Fig. 1. Gel Analysis of Total RNA isolated from cells and tissues
Total RNA was purified from different cells and tissues using the RNA-spin™ Total RNA Extraction Kit for Cell/Tissue. And then total RNA was analyzed in gel electrophoresis. 1㎕ of eluted solution was loaded per lane on a 1.0% agarose gel.
Lane 1, SNU 5(1x 10^6 cells); **lane 2,** B16(1x 10^6 cells); **lane 3,** spleen; **lane 4,** Kidney

• *Comparision of RT-PCR amplification of total RNA from different company kit*

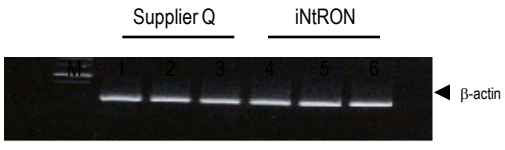

Fig. 2. RT-PCR Amplification for β-actin gene with ONE-STEP RT-PCR PreMix Kit of iNtRON
Total RNA was purified from cell using RNA-spin™ Total RNA Extraction Kit for Cell/Tissue. And then, the first strand cDNA was synthesized and its PCR reaction using ONE-STEP RT-PCR PreMix Kit.
Lane 1-3, supplier Q; **lane 4-6**, iNtRON
Lane M, marker DNA; **lane 1,4,** SNU 5; **Lane 2,5,** K562; **lane 3,6,** B16

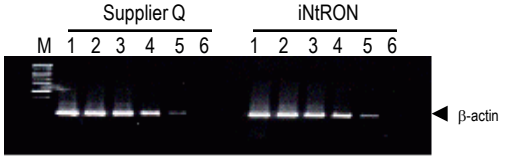

Fig. 3. RT-PCR Amplification for β-actin gene with ONE-STEP RT-PCR PreMix Kit of iNtRON
Total RNA was purified from cell using RNA-spin™ Total RNA Extraction Kit. And then, the first strand cDNA was synthesized and its PCR reaction using ONE-STEP RT-PCR PreMix Kit.
Lane M, marker DNA; **lane 1,** undiluted RNA; **lane 2,** 10^{-1} diluted RNA; **lane 3,** 10^{-2} diluted RNA; **lane 4,** 10^{-3} diluted RNA; **lane 5,** 10^{-4} diluted RNA; **lane 6,** 10^{-5} diluted RNA

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommendation
Low RNA yield or no RNA	Too much starting material	- Check the step 1, 2 of protocol I, III; 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	- Check the "Note for before using RNA-spin™ Kit"; Use DEPC-treated glassware and wear gloves at all time.
DNA contamination		- Check the "Note for before using RNA-spin™ Kit"; If genomic DNA remaining can be removed using RNase-free DNase by a DNase digestion after RNA purification.
Lysate too viscous to pipet easily	Initial lysate too viscous	- Check the step 1, 2 of protocol I, III; Too much starting material. Dilute lysate with R-buffer.
RNA does not perform well in the downstream application	Ethanol carryover	- Ensure that during the Washing buffer B, the RNA-spin™ column is spun at maximum speed 1min to dry the RNA-spin™ column

RELATED PRODUCTS

Product Name	Cat.No.
easy-spin™ Total RNA Extraction Kit	17221
ONE-STEP RT-PCR PreMix	25101
easy-BLUE™ Total RNA Extraction Kit	17061
Viral Gene-spin™ Viral DNA/RNA Extraction Kit	17151