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

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 RA. So, RNA-spin™ buffer system is optimized to allow rapid and simple cell lysis followed by selective binding of RA 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 10 <-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 $1x10^7$ 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 RA 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 350 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 ?-mercaptoethanol is added to R-buffer. The 350 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 350 (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 800 . For larger volume, sample reload and spin again.
5. Add 700 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 700 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 50 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 175 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 .
   [**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 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 600 of prepared R-buffer to the supernatant and vigorously vortex.
   Note : Before use, must be add 10 <-mercaptoethanol per 1ml R-buffer.
7. Add 430 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 10 of <-mercaptoethanol per 1ml R-buffer. The 350 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 4C, and carefully transfer the supernatant to a new 1.5ml tube (not provided) by pipetting.
5. Proceed to step 3 in Protocol I.