Total RNA Isolation Kit

User's Instruction

Introduction

The Total RNA Isolation Kit takes the advantage of both Redzol and SiMax™ membrane spin column. It provides a fast and simple technique for the preparation of purified intact total RNA from tissues and cultured cells in as short as 45 minutes, free of DNA and protein contamination. The purity and yield are superior to Redzol alone. Each spin column can process 50~100 mg of tissues or 5~10×10^6 cultured cells. The purity and integrity of RNA isolated from tissues or cultured cells with the Total RNA Isolation Kit can meet the experimental need, such as reverse transcriptase PCR (RT-PCR), RNase protection assays, primer extension, Northern blot analysis, oligo(dT) selection of poly(A)+ mRNA, in vitro translation and cDNA library construction.

Package and Components

	50 preps
Redzol reagent	50 ml
SiMax™ membrane spin column	50 sets
RNA washing buffer	40 ml
DEPC water	3 ml

Precautions

1. Replace new gloves regularly, as skin and labware may have RNases.
2. Treat tips with DEPC or use RNase-free tips to avoid cross-contamination.
3. Treat plastic and glass wares with DEPC or use RNase-free vessels.

Procedures

1. Preparation

Estimate the amount of tissues and cells. 1 ml Redzol is needed for 50~100 mg tissue or 5~10×10^6 cells.

2. Lysis of tissues or cells

1) Adhesive cells

Take out growth media. Add Redzol. Pipette up and down for several times with pipettor to ensure all cells lyse. Then transfer the lysate into a new centrifuge tube. 1 ml Redzol is needed to lyse 10 cm^2 cells.

2) Suspension cells

Collect cells after centrifuge, discard liquid. Add Redzol. Pipette up and down for several times with micropipette to ensure all cells lyse (some yeast and bacteria cells need grinder to help them lyse). Then transfer the lysate into a new centrifuge tube. Add 1 ml Redzol into every 5~10×10^6 cells of animal, plant and yeast cells or into 1×10^7 bacteria cells.

[NOTE] Avoid washing cells before adding Redzol as it may increase mRNA degradation.

3) Tissues

Cut tissues into little pieces and put it into a homogenizer. Add Redzol and homogenize the tissues. Then transfer the mixture into a new centrifuge tube. Alternatively, grind tissues into powder in liquid nitrogen. Then add 1ml Redzol into every 50~100 mg tissues to lyse.

[NOTE] The total volume of the tissue should be under 10% of the Redzol volume.

3. Phase Separation

1) Stand the homogenate at 15~30℃ for 5 min to lyse completely.

2) Add 0.2ml $CHCl_3$ into 1 ml Redzol. Vortex and stand at 15~30℃ for 2~3 min.

3) Centrifuge at 12,000 rpm for 15 min. The mixture is divided into three phases, RNA is in the top water phase.

4. Purification on-column

1) Transfer the top water phase into a new 1.5 ml centrifuge tube, add 200 µl ethanol. Mix gently by reverse the tube several times.

2) Transfer the mixture into a SiMax™ membrane centrifuge column. Stand for at least 3 min and centrifuge for 2~3 min at 12,000 rpm.

3) Discard the flow-through. Place the SiMax™ membrane centrifuge column into the collection tube. Add 600 µl RNA washing buffer and centrifuge at 12,000 rpm for 1 min. Discard the flow-through.

4) Centrifuge at 12,000 rpm for 2 min again, place the SiMax™ membrane centrifuge column into a new 1.5 ml microtube. Add 50 µl DEPC water or 0.5% SDS into the center of the column. Stand for 1~2 min. Centrifuge at 12,000 rpm for 1 min to collect the elution.

5) Elute again to increase the yield of RNA.