ShinePrep® RNA Miniprep Kits for qPCR User Manual

Cat.Nos.ZN00701(50 rxns)

ZN00702(100 rxns)

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Ⅰ、Description

The ShinePrep RNA miniprep kit provides a rapid method for purification of high-quality total RNA from small samples of tissue or cultured cells.Extreme purity of RNA is critical because contaminating DNA present in the RNA sample can give rise to amplification products that mimic the amplification product expected from the RNA target. The ShinePrep RNA miniprep kit eliminates these problems by providing high yields of total RNA with undetectable levels of DNA from animal tissues and cultured cells. This simple and effective method of RNA purification eliminates time-consuming ethanol precipitations used in other RNA purification methods. The ShinePrep RNA method employs a spin cup with a silica-based fiber matrix that binds RNA in the presence of a chaotropic salt while a series of washes removes contaminants. The lysis buffer contains guanidine thiocyanate, one of the strongest protein denaturants, to lyse the cells and to prevent RNA degradation by ribonucleases (RNases). The supernatant is then transferred to a spin cup with a silicabased fiber matrix where the RNA binds to the fiber matrix. Treatment with a low-salt wash buffer and digestion with DNase removes the remaining DNA. A series of washes removes the DNase and other proteins. Highly pure RNA is eluted from the fiber matrix with a small volume of low-ionicstrength buffer and captured in a microcentrifuge tube. The highly pure RNA is ideal for conventional RT-PCR and real time quantitative RT-PCR and is suitable for cDNA synthesis, RT-PCR, northern blotting, Rnase protection assay, and primer extension analysis.

Ⅱ、Additional Materials Required

Diethylpyrocarbonate (DEPC)
chloroform
Isopropyl Alcohol
Homogenizer

Ⅲ、Component

Components	ZN00701 (50 Preps)	ZN00702 (100 Preps)
SG Spin Column	50	100
Collection tube	50	100
LS	50ml	100ml
WS	50ml	100ml
RNase-free H2O	2ml	4ml

Ⅳ、Shipping and Storage

The kit is stable for 12 months at room temperature. For longer storage, keep all contents of the kit cold.

Ⅴ、Protocol

- **Procedure for Isolation of Total RNA from Tissue, Plant Cells and Filamentous Fungi**

1.Samples Preparation:Grind sample under liquid nitrogen to a fine powder using a mortar or

pestle. Transfer the mixture of tissue powder and liquid nitrogen to 2.0 ml microtube and allow the liquid nitrogen to evaporate.Do not allow the sample to thaw. Continue immediately with Step2.

2. Add 1000ul of LS Solution to a maximum of 50 mg of tissue powder and vortex vigorously.

3. Add 200 ul of chloroform to the mixture (step 2). Mix gently.

4. centrifuge at 12,000rpm for 5 minute.

5.Place a Spin column to 2.0-ml collection tube and transfer the supernatant to the column spin,in the meantime add 200ul Isopropyl Alcohol to the column,mix gently.

6. centrifuge at 12,000rpm for 3 minute. Discard flow-through and place the column back to the same collection tube.

7. Add 500ul of WS Solution to the column, centrifuge at 12,000rpm for 2 minute, and discard flow-though and place the column back to the same collection tube. spin once more to remove the residue of WS Solution.

8. Transfer the column to a clean RNase-free 1.5 ml microtube, add 30-50ul of RNase-free H2O onto the center part of the membrane in the column, and incubate at 50℃ for 2 minutes and spin down at 10,000rpm for 1 minute. Keep RNA sample at –70℃

- **Procedure for Isolation of Total RNA from Animal Cells.**

1. Samples Preparation:

A. Harvest cells

(a) Cells grow in suspension: Determine the number of cells. Spin down the appropriate number of cells for 5 minutes at 1,200rpm in a RNase-free microtube. Carefully remove all supernatant by aspiration, and continue with Step 2 of the protocol.

(b) Cells grown in a monolayer. Determine the number of cells. Cells grown in a monolayer in cell-culture vessels can be trypsinized. Aspirate medium, and wash cells with PBS. Aspirate PBS and add 0.1-0.25% trypsin in PBS. After cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer cells to 1.5-ml RNase A-free microtube, spin by centrifugation at 1,200rpm for 5 minutes. Completely aspirate supernatant, and continue with Step 2 of the protocol.

Note: Incomplete removal of the cell-culture will inhibit lysis and dilute the lysate, affecting the conditions for binding RNA to Spin column. Both effects may reduce RNA yield.

B. Disrupt cells by addition of LS Solution

(a) For pelleted cells Loosen cell pellet by flicking the tube and add LS Solution

(b) For monolayer cells. Add LS Solution to monolayer cells (according to table below).Cellect cell lysate with a rubber policeman.Vortex.No cell clumps should be visible before proceeding to Step 2

LS Solution (μl)	Number of pelleted cells	Dish diameter (cm)
500	> 5×10^6	6
1000	> $5 \times 10^6$to 1×10^7	6-10

2. Add 200 ul of chloroform to the mixture (step 1). Mix gently. Transfer the mixture to 2.0ml microtube.

3. centrifuge at 12,000rpm for 5 minute.

4.Place a Spin column to 2.0-ml collection tube and transfer the supernatant to the column spin,in the meantime add 200ul Isopropyl Alcohol to the column,mix gently.

6. centrifuge at 12,000rpm for 3 minute. Discard flow-through and place the column back to the same collection tube.

7. Add 500ul of WS Solution to the column, centrifuge at 12,000rpm for 2 minute, and discard flow-though and place the column back to the same collection tube. spin once more to remove the residue of WS Solution.

8. Transfer the column to a clean RNase-free 1.5 ml microtube, add 30-50ul of RNase-free H2O onto the center part of the membrane in the column, and incubate at 50℃ for 2 minutes and spin down at 10,000rpm for 1 minute. Keep RNA sample at -70℃

- **Procedure for Isolation of Total RNA from Bacteria**

1 Samples Preparation: Harvest the appropriate number of cells ($<1\times10^9$) by centrifugation at 1000rpm for 3 minutes at 4℃.Discard supernatant ensuring all media is completely removed.

2. Add 1000ul of LS Solution to the precipitation and vortex vigorously.

3. Add 200 ul of chloroform to the mixture (step 2). Mix gently.

4. centrifuge at 12,000rpm for 5 minute.

5.Place a Spin column to 2.0-ml collection tube and transfer the supernatant to the column spin,in the meantime add 200ul Isopropyl Alcohol to the column,mix gently.

6. centrifuge at 12,000rpm for 3 minute. Discard flow-through and place the column back to the same collection tube.

7. Add 500ul of WS Solution to the column, centrifuge at 12,000rpm for 2 minute, and discard flow-though and place the column back to the same collection tube. spin once more to remove the residue of WS Solution.

8. Transfer the column to a clean RNase-free 1.5 ml microtube, add 30-50ul of RNase-free H2O onto the center part of the membrane in the column, and incubate at 50℃ for 2 minutes and spin down at 10,000rpm for 1 minute. Keep RNA sample at –70℃

VI、Related Products

EnergicScript® First Strand cDNA Synthesis Kits

Cat.Nos.ZK00804(50 rxns×20ul)

ZK00805(100 rxns×20ul)

ShineProbe® Real Time qPCR Kits

Cat.Nos.ZK00713(50 rxns×50ul)

ZK00714(100 rxns×50ul)

FicoScript® M MLV Reverse Transcriptase

Cat.Nos.ZP00601(1000 U)

ZP00602(5000 U)

PowerQ® Taq plymerase

Cat.Nos.ZP00102(1000 U)

ZP00103(5000 U)

ShinePolo® One step RT-PCR qPCR Kits

Cat.Nos.ZK00102(50 rxns×30ul)

ZK00103(100 rxns×30ul)

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