Troubleshooting Guide

Problem	Possible cause	Suggested solution
Low yield	Sample not homogenized completely	Be sure to incubate for 5~10 minutes at room temperature after lysis. Confirm the completely homogenized sample in buffer LYS.
	Too much starting sample	Reduce the amount of starting sample. Especially tissue sample, use the correct amount of starting sample.
	Poor quality of starting material	Process the sample immediately after harvest from animal. To process later, freeze the tissue rapidly in liquid nitrogen.
	Culture media not completely removed	Remaining culture media affect lysis and binding condition of buffer LYS.
	Centrifugation at high speed in lysis step	Do not centrifuge over 3000 rpm in the lysis step.
Column clogging	Sample not homogenized completely	Be sure to incubate for 5~10 minutes at room temperature after lysis. Confirm the completely lysed sample in buffer LYS.
	Too much starting sample	Reduce the amount of starting sample. Especially tissue sample, use the correct amount of starting sample.
RNA degradation	Sample manipulated too much before process	Process the sample immediately after harvest from animal. For cultured cell, minimize washing steps.
	Improper storage of RNA	Store isolated RNA at -70°C, Do not store at -20°C.
	Reagent or disposable is not RNase-free	Make sure to use RNase free products only.
DNA contamination	No treatment of buffer GW1	Follow the Ribospin™ manual. See step 6.
	No DNase treatment	Refer to the optional procedure.
Eluate does not perform well in downstream application	Residual ethanol remains in eluate	To remove any residual ethanol included in buffer RNW from mini spin column membrane, Centrifuge again. See step 10.

GeneAll®

For research use only

Ribospin™

For tissue and cell sample

Cat. No. 304-150

Size: 50 prep

Kit Contents

Components	Quantity	Storage
Buffer LYS	30 ml	
Buffer GW1	35 ml	
Buffer RNW	50 ml	
RNase-free water	15 ml	Room temperature
GeneAll Column type F	50	
2 ml collection tube	50	
1.5 ml collection tube	50	

Product Specifications

Specification	Ribospin™
Type	Spin
Maximum amount of starting samples	~ 25 mg or ~ 5 x 10^6 cells
Maximum loading volume of spin column	~ 750 ul
Minimum elution volume	~ 40 ul
Maximum binding capacity	~ 100 ug

Quality Control

Ribospin™ is manufactured in strictly clean condition, and its degree of cleanness is monitored periodically. For consistency of product, the quality certification process is carried out from lot to lot thoroughly and only the qualified is approved to be delivered.

Storage Conditions

Ribospin™ should be stored at room temperature (15 ~ 25°C). All components are stable for 1 year.

Precautions

The buffers included in Ribospin™ contain irritant which is harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protector, and follow standard safety precautions. In case of contact, wash immediately with plenty of water and seek medical advice.

Buffer LYS and GW1 contain chaotropes. It can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solutions directly to the sample-preparation waste.

Preventing RNase Contamination

RNase can be introduced accidentally into a RNA preparation. Wear disposable gloves always, because skin often contains bacteria that can be a source of RNase. Use sterile, disposable plasticwares and automatic pipettes reserved for RNA work to prevent cross-contamination with RNase on shared equipment.

Product description

Ribospin™ provides a convenient method for isolation of total RNA from cell and tissue samples. Ribospin™ procedures employed the glassfiber membrane technology for the fastest and the most convenient of high purity RNA isolation, instead of conventional alcohol precipitation or phenol / chloroform extraction. Ribospin™ buffer system provides the effective binding condition of RNA to glassfiber membrane and minimizes the contamination of DNA and impurities.

In lysis step, cell and tissue samples are homogenized in buffer LYS. Then the lysate is directly applied to mini spin column and RNA binds to mini spin column through centrifugation. The remained DNA and impurities on the membrane are washed away by two different wash buffers. At last, pure RNA is eluted by nuclease-free water. Whole procedure takes only 15 minutes and the eluate is suitable for RT-PCR or any downstream application without further manipulation. Ribospin™ procedure should be performed at room temperature. The eluate should be treated with care because RNA is very sensitive to contaminants, such as RNases, often found on general labware and dust. To ensure RNA-stability, it is recommended to store at 4°C for immediate analysis or to freeze at -70°C for long-term storage.

Protocol for Ribospin™

1. Harvest samples in a tube.

Cell samples

- Cells grown in Monolayer

  Harvest the 5 x 10^6 cells carefully using scraper. And pellet cells by centrifugation at low speed (below 3000 rpm) for 5 minutes, then discard the culture medium.

- Cells grown in suspension

  Pellet 5 x 10^6 cells by centrifugation at low speed (below 3000 rpm) for 5 minutes, then discard the culture medium.

  * Do not wash cells before lysing with buffer LYS as this may contribute to mRNA degradation.

Tissue samples

Harvest ~ 25 mg tissue samples in a tube. For later use, the recommended sample handling is to put prepared tissue directly into tissue storage buffer or to freeze the tissue rapidly in liquid nitrogen.

2. Add 400 ul of buffer LYS to the tube and lyse the sample by pipetting or homogenizing.

Add 10 ul β-mercaptoethanol per 1 ml buffer LYS.

Cell samples

Lyse 5 x 10^6 cells in 400 ul buffer LYS with pipetting. Insufficient lysis may result in low RNA recovery or column clogging.

Tissue samples

Homogenize ~ 25 mg of tissue samples in 400 ul buffer LYS using homogenizer. Thoroughly disrupt the tissue in buffer LYS. Not clarified sample may cause clogging of the column in subsequent steps.

Caution!

Buffer LYS occasionally generate foam because of included detergent. We recommend careful proceeding. In the case of foaminess, spin the tube at low speed (below 3000 rpm) for 2 minutes, then transfer the supernatant to a fresh tube.

* Do not centrifuge over 3000 rpm in lysis step.

3. Incubate the lysate for 5 minutes at room temperature.

In case of tissue samples, incubate the lysate for 10 minutes at room temperature.

4. Transfer the lysate to a mini spin column.

5. Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.

Discard the pass-through and reinsert the mini spin column back into the same tube.

6. Add 500 ul of buffer GW1 to the mini spin column.

7. Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.

Discard the pass-through and reinsert the mini spin column back into the same tube.

8. Add 700 ul of buffer RNW to the mini spin column.

9. Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.

Discard the pass-through and reinsert the mini spin column back into the same tube.

10. Centrifuge at ≥ 10,000 x g for an additional 1 minute at room temperature to remove residual wash buffer. Transfer the mini spin column to a new 1.5 ml microcentrifuge tube (provided).

Residual ethanol may interfere in downstream reactions. Care must be taken at this step for eliminating the carryover of buffer RNW.

11. Add 50 ul of RNase-free water to the center of the membrane in the mini spin column. Let it stand for 1 minute.

12. Centrifuge at ≥ 10,000 x g for 1 minute at room temperature.

Purified RNA can be stored at 4°C for immediate analysis and can be stored at -70°C for long term storage.

(*Option*) DNA-free RNA procedure using DNase I and Riboclear™

• Need for optional experiment

Riboclear™ (cat. 303-150)

DNase I (Bioshop® *cat. DRB003* or equivalent)

Optional procedure is needed to get DNA-free RNA. Ribospin™ procedure efficiently removes most of DNA. However, further DNA removal may be needed for some applications, such as RT-PCR which is interfered by small amount DNA. In this case, DNase I is effective to digest contaminating DNA in RNA eluent. But, after activation, DNase I must be removed from RNA eluent because it could degrade DNA made in the reverse transcription or PCR. Riboclear™ provides RNA clean up method for simple and effective removal of impurities including DNase I.

Method

1. Mix RNA eluent with DNase I and buffer.

DNase I is sensitive to physical denaturation.

Careful pipetting is required. To use DNase I appropriately, follow product manual of DNase I.

2. Incubate the mixture for 10 minutes at room temperature.

3. Follow the Riboclear™ manual.

To confirm Riboclear™ manual, check the below web site.

http://www.geneall.com/english/product_RNA_clup.html

Brief protocol

Homogenization

Homogenize ~ 25 mg tissue or ~ 5 x 10^6 cells.

Incubate the homogenate for 5 minutes at RT

RNA bind

Transfer the lysate to a mini spin column and centrifuge at ≥ 10,000 x g for 30 seconds.

RNA wash

Add 500 ul of buffer GW1 and centrifuge at ≥ 10,000 x g for 30 seconds.

Add 700 ul of buffer RNW and centrifuge at ≥ 10,000 x g for 30 seconds.

Centrifuge at ≥ 10,000 x g for an additional 1 minute.

RNA elution

Add 50 ul of RNase-free water.

Let it stand for 1 minute.

Centrifuge at ≥ 10,000 x g for 1 minute.

Pure RNA

The yield of total RNA may vary depending on the tissue or cells from which it is obtained.

	Sample type	Amount of starting material	Yield of Total RNA
Cultrued cell	CHO	1 x 10^6	~ 15 ug
	RAW 264.7	1 x 10^6	~ 20 ug
Tissue	Liver	10 mg	~ 60 ug
	Kidney	10 mg	~ 30 ug
	Spleen	10 mg	~ 35 ug
E. coli	DH5α	O.D 1.5 (2ml pellet)	~ 10 ug