Troubleshooting Guide

Problem	Possible cause	Suggested solution
Low yield of RNA	RNA not completely solubilized	To increase solubilization, pipette RNA pellet repeatedly in SDS or DEPC-treated water. Heat sample at 55ºC for 10 to 15 minutes.
		Do not allow RNA pellet to dry completely.
		Do not lyophilize or vacuum-dry samples.
		Do not centrifuge RNA above 12,000 x g.
	Sample not homogenized completely	Make sure no particulate matter remains. Be sure to incubate for 5 minutes at room temperature after homogenization.
	Some aqueous phase left	Perform second extraction with the remaining aqueous phase.
	Short precipitation time	Extend the precipitation time. Precipitate for 30 minutes overnight at -20ºC
Degraded RNA	Sample manipulated too much before RiboEx™ addition	Process tissue immediately after harvest from animal.
		For cultured cell, minimize washing steps. Add RiboEx™ directly to plates. Do not trypsinize cells.
	Improper storage of RNA	Store isolated RNA at -70ºC, Do not stored at -20ºC.
	Frozen tissue thawed in absence of RiboEx™	Thaw the frozen sample directly in RiboEx™.
	Aqueous solution or the tubes used for experiment may not have been RNase free	Make sure to use RNase free products only.
	Formaldehyde used for the agarose gel electrophoresis may have a pH value below 3.5	Check the pH of the formaldehyde solution.
Low $A_{260/280}$ (<1.6)	Residual organic solvents (phenol, chloroform) in the RNA rehydrates	Be sure not to carry any of the organic phase with the RNA sample.
		Precipitate the RNA again with ethanol.
	Sample not completely homogenized with RiboEx™	Use 0.5 ml RiboEx™ for up to 50 mg tissue or 10^6 cells.
		Be sure to incubate sample for 5 minutes at room temperature after homogenization.
	pH of solution is acidic	Dissolve sample in TE or 1 mM sodium phosphate buffer pH 8.0 instead of water.
	A_{260} or A_{280} outside the linear range	Dilute sample to bring absorbency into linear range.
DNA contamination	Part of the interphase was removed with the aqueous phase	Be sure not to take any of the interphase (contains the DNA) with the aqueous phase.
	Insufficient RiboEx™ used	Use 0.5 ml RiboEx™ for 50 mg tissue or 10^6 cells.
	Cells or tissue contained organic solvents	Be sure that original sample does not contain organic solvents such as ethanol or DMSO.
	Insoluble materials were not removed before chloroform extraction	Remove any particulate material before chloroform addition. This material may trap DNA.
Colored aqueous phase containing RNA	For tissue with high fat content (eg, skin), fat micelles did not completely separate to top of aqueous phase during centrifugation	Centrifuge sample before addition of chloroform, and remove fat layer on top.
	Aqueous phase turns yellow upon addition of isopropyl alcohol	Try a fresh bottle of isopropyl alcohol. This color has inhibited RT-PCR.
Cells not detached completely from flask after addition of RiboEx™	This can be seen with some strongly adherent cells	After addition of RiboEx™, let cells sit 2 to 3 minutes. Scrape cells with a rubber policeman. Incubate for several minutes. Collect and repeatedly pipette cells over flask surface. Then transfer homogenate to a tube.
Precipitate in bottom of the tube after addition of chloroform	High amount of polysaccharides or proteoglycans	Centrifuge sample before addition of chloroform. Add 0.4 ml of isopropyl alcohol and 0.1 ml of a high salt precipitation solution to the aqueous phase.

GENEALL BIOTECHNOLOGY CO., LTD www.geneall.com T : 82-2-407-0096 F : 82-2-407-0779

GeneAll®

For research use only

RiboEx™

Total RNA isolation solution

Cat. No. 301-001

Store at 4℃ Size: 100 ml

Quality Control

RiboEx™ is manufactured in strict clean condition, and its degree of cleanness is monitored periodically.

For quality control, the quality certification process is carried out thoroughly and only the qualified is delivered.

Storage conditions

Store at 2 to 8°C for optimal performance.

Precautions

RiboEx™ contains phenol, which is poisonous, and guanidine salt, which is an irritant, therefore, when working with RiboEx™, use gloves and eye protection, avoid contact with skin or clothing, and avoid inhaling vapor. In case of contact, wash immediately with plenty of water and seek medical advice.

Materials Not Provided

For RNA isolation

* Nuclease-free Water
* Equipment for homogenizing solid tissue
* RNase-free centrifuge tubes
* Chloroform or 1-bromo-3-chloropropane (BCP)
* 100% isopropanol, ACS grade or better
* 100% ethanol, ACS grade or better
* High salt precipitation solution for plant (0.8 M sodium citrate and1.2 M NaCl)

Precautions for preventing RNase Contamination

RNase can be introduced accidentally into the RNA preparation. Therefore, always wear disposablegloves.

Because skin often contains bacteria that can bea source of RNase. Use sterile, disposable plasticware and automatic pipettes reserved for RNA work to prevent cross-contamination with RNase from shared equipment. Also, Keep the RNA preparation covered to prevent dust.

Because dust is the one of the major sources of RNase contamination.

Product Description

RiboEx™ is a complete kit with ready-to-use reagent for the isolation of total RNA from samples of human, animal, plant, yeast, or bacterial and viral origin. RiboEx™ is based on disruption of cells in guanidine salt/detergent solution, followed by organic extraction and alcohol precipitation of the RNA, and which allows simultaneous processing of a large number of samples.

RiboEx™ can yield up to 10 ug / mg tissue or up to 22 ug / 1 x 10^7 cultured cells of highly purified total RNA.

The resulting total RNA is suitable for the isolation of Poly A^+ RNA or for Northern blotting, Dot blotting, *in vitro* Translation, cloning, RT-PCR, RNase protection assays, and other analytical procedures.

Protocol for RNA isolation

1. Homogenize 50 ~ 100 mg tissue samples in 1 ml RiboEx™. Homogenize 5 ~ 10 x 10^6 cells in 1 ml RiboEx™.

Tissue samples

Homogenize tissue samples in 1 ml RiboEx™ per 50 ~ 100 mg tissue using homogenizer. The sample volume should not exceed 10 % of the volume of RiboEx™ used for homogenization.

Handling fresh tissue

Immediately after dissection, inactivate RNases by any one of the following treatments.

* Homogenize in RiboEx™ immediately.
* Freeze rapidly in liquid nitrogen.
* Submerge in a tissue storage buffer to protect RNA from RNase.

Cell samples

Cells grown in Monolayer

Pour off media, add 1 ml of RiboEx™ per 10 cm^2 of culture dish area. Pass the cell lysate several times through a pipette.

An insufficient amount of RiboEx™ may result in contamination of the isolated RNA with DNA.

Cells grown in suspension

Pellet cells by centrifugation, then lyse in 1 ml of RiboEx™ per 5 ~ 10 x 10^6 animal, plant, or yeast cells, or per 10^7 bacterial cells, by repetitive pipetting or vortexing.

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

2. Incubate the homogenate for 5 minutes at room temperature.

This step allows nucleoprotein complexes to completely dissociate. Homogenized samples can be stored at -70ºC for at least one month.

3. *(optional)* Centrifuge at 12,000 x g for 10 minutes at 4ºC and transfer the supernatant to a fresh tube.

This optional step is only required for homogenate with high contents of proteins, fats, polysaccharides or extracellular materials such as muscles, fat, tissue, and tuberous parts of plants. The resulting pellet contains extracellular membranes, and high molecular weight DNA, while the supernatant contains RNA. Fat tissue samples will form a layer on top of the aqueous phase, therefore, remove and discard this layer.

4. Add 0.2 ml of chloroform per 1 ml of RiboEx™. Shake vigorously for 15 seconds, store for 2 minutes at room temperature.

Alternatively, 0.1 ml of BCP (1-bromo-3-chloropropane) can be used in place of chloroform.

5. Centrifuge at 12,000 x g for 15 minutes at 4ºC, then transfer the aqueous phase to a fresh tube.

The mixture separates into a lower layer, an interphase, and a colorless upper aqueous layer. The upper aqueous layer is about 50 % of the volume of RiboEx™ used for homogenization.

Centrifugation at above 8ºC may cause some DNA to partition in the aqueous phase.

6. Add 0.5 ml of isopropyl alcohol per 1 ml of RiboEx™ used for the initial homogenization and gently mix the solution by inverting, 3 ~ 5 times.

Proteoglycan and polysaccharide contamination

To RNA precipitate from tissue with high content of proteoglycans and/or polysaccharides (after step 5), these contaminating compounds from the isolated RNA are removed by the modified method.

Add to the aqueous phase 0.4 ml of isopropyl alcohol and 0.1 ml of a high salt precipitation solution (0.8 M sodium citrate and 1.2 M NaCl) per 1 ml RiboEx™. After mixing this solution, proceed with the step 7.

This modified precipitation effectively precipitates RNA and maintains proteoglycans and polysaccharides in a soluble form. This procedure should only be used if the sample is known to have a high content of proteoglycans and polysaccharides. To isolate pure RNA from plant material containing a very high level of polysaccharides, the modified precipitation should be combined with an additional centrifugation of the initial homogenate.

7. Incubate samples for 10 minutes at room temperature.

8. Centrifuge at 12,000 x g for 10 minutes at 4ºC, and discard the supernatant.

Carefully remove the supernatant without disturbing the pellet.

Precipitated RNA forms a gel-like or white pellet on the side and bottom of the tube. To increase yield, store sample for 30 minutes ~ overnight at -20ºC.

9. Add 1ml of 75 % ethanol per 1ml RiboEx™ to wash the RNA pellet.

The RNA precipitate can be stored in 75 % ethanol at 4ºC for one week, or at -20ºC for at least one year.

10. Centrifuge at 7,500 x g for 5 minutes. Carefully discard the supernatant, ethanol, and air-dry the RNA pellet for 5 minutes.

The RNA pellet is very loose at this point and care must be taken to avoid missing the pellet.

Do not completely dry the RNA pellet as this will greatly decrease its solubility.

Ethanol should be completely removed to perform perfect downstream application.

11. Dissolve RNA in DEPC-treated water or in 0.5 % SDS solution by incubating for 10 ~ 15 minutes at 56ºC.

The resuspension volume is applied to samples. For example, enough resolution volume is 50 ~ 100 ul per 1 ml reaction for *E. coli*, cultured cell, or plant, or 300 ~ 500 ul per 1 ml reaction for tissue.

For immediate analysis, store at 4ºC and for long term storage, store at -70ºC.

For best results in RT-PCR, dissolve the RNA in DEPC-treated water not included EDTA.

The final precipitation of total RNA will be free of DNA and proteins, and will have a $O.D_{260/280}$ ratio of 1.8 to 2.1.

The yield of total RNA

Sample type	Amount of starting material	Yield of Total RNA
Liver, Spleen	1 mg	~ 10 ug
Kidney	1 mg	~ 4 ug
Brain	1 mg	~ 1.5 ug
CHO cell	1.5 x 10^6	~ 20 ug
E. coli	$O.D_{600}$ = 1.8 (1,5 ml pellet)	~ 60 ug

Brief protocol



Homogenization

Homogenize 50 ~ 100 mg / ml tissue samples or 1 x 10^7 cells.
Incubate the homogenate for 5 min at R.T.





Phase separation

Add 0.2 ml chloroform Incubate the mixture for 2 min at R.T.
Centrifuge at 12,000 x g for 10 min at 4ºC, then transfer the aqueous phase to a fresh tube.





RNA precipitation

Add 0.5 ml isopropyl alcohol per 1 ml starting solution.
Incubate the mixture for 10 min at R.T.
Centrifuge at 12,000 x g for 10 min at 4ºC. Discard the supernatant.





RNA wash

Add 1 ml of 75 % ethanol per 1 ml starting solution.
Centrifuge at 7,500 x g for 5min at 4ºC.
Briefly air-dry the RNA pellet.

RNA solubilization

Dissolve RNA in RNase-free water.
Incubate for 10 min at 56ºC.