Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low yield of RNA	RNA not completely solubilized	To increase solubilization, pipette RNA pellet repeatedly in 0.5% SDS solution or DEPC-treated water. Heat sample at 55ºC for 10 to 15 min.
		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 min 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 min 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}/A_{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 (step 9).
	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 min 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 absolute isopropanol	Try a fresh bottle of absolute isopropanol. 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 min. 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 absolute isopropanol and 0.1 ml of a high salt precipitation solution to the aqueous phase.

Ver 1.1

GeneAll®

For research use only

RiboEx™

Total RNA isolation solution

Cat. No. 301-001
Size : 100 prep
Store at 4℃

Quality Control

RiboEx™ is manufactured in strictly clean conditions, and its degree of cleanness is monitored periodically.
Quality control is carried out thoroughly from lot to lot, and only the qualified products are approved to be delivered.

Storage Conditions

RiboEx™ should be stored at 2 to 8°C for optimal performance.

Safety Information

RiboEx™ contains phenol and guanidine salt which are harmful as an irritant when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken when handling such material. Always wear gloves and eye protection, and follow standard safety precautions.

Prevention of RNase Contamination

RNase can be introduced accidentally during RNA purification. Wear disposable gloves always, because skin often contains bacteria and molds that can be a source of RNase contamination. Use sterile, disposable plastic wares and automatic pipettes to prevent cross-contamination of RNase from shared equipment.

Materials Not Provided

* DEPC-treated water or 0.5% SDS solution
* Chloroform or 1-bromo-3-chloropropane (BCP)
* Absolute isopropanol (ACS grade or better)
* 75% ethanol (ACS grade or better)
* High salt precipitation solution (0.8 M sodium citrate and 1.2 M NaCl)

Product Disclaimer

RiboEx™ is for research use only, not for use in diagnostic procedure.

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 μg/mg tissue or up to 22 μg/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 min 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 min 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 sec, store for 2 min 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 min 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 absolute isopropanol 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 precipitate RNA from tissues containing high contents of proteoglycans and/or polysaccharides (after step 5), the modified method for precipitation step should be required. This modified precipitation effectively isolates pure RNA eliminating proteoglycans and polysaccharides.

   1) Add 0.4 ml of absolute isopropanol and 0.1 ml of a high salt precipitation solution (0.8 M sodium citrate and 1.2 M NaCl) to the aqueous phase.

   2) Mix the solution by inverting and proceed with the step 7.

7. **Incubate the mixture for 10 min at room temperature.**

   To increase yield, store the mixture for 30 min~overnight at -20ºC.

8. **Centrifuge at 12,000 x g for 10 min 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.

9. **Add 1 ml of 75% ethanol 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 min at 4ºC. Carefully discard the ethanol and air-dry the RNA pellet for 5 min.**

    Care must be taken to avoid missing the RNA pellet.

    Do not completely dry the RNA pellet leading to decrease of 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 min at 56ºC.**

    The resuspension volume is based on the species and conditions of starting sample or the downstream applications. For example, enough volume to resuspend the RNA pellet is 50~100 µl for E. coli, cultured cell, or plant, or 300~500 µl 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 purified total RNA will be free of DNA and proteins, and will have A_{260}/A_{280} ratio of 1.8 to 2.1.

The typical yield of total RNA

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

Brief protocol

Homogenization

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

Incubate the homogenate for 5 min at RT.

Phase separation

Add 0.2 ml chloroform.

Incubate the mixture for 2 min at RT. Centrifuge at 12,000 x g for 15 min at 4ºC, then transfer the aqueous phase to a fresh tube.

RNA precipitation

Add 0.5 ml absolute isopropanol.

Incubate the mixture for 10 min at RT. Centrifuge at 12,000 x g for 10 min at 4ºC. Discard the supernatant.

RNA wash

Add 1 ml of 75% ethanol.

Centrifuge at 7,500 x g for 5 min at 4ºC. Briefly air-dry the RNA pellet.

RNA solubilization

Dissolve RNA in DEPC-treated water.

Incubate for 10 min at 56ºC.