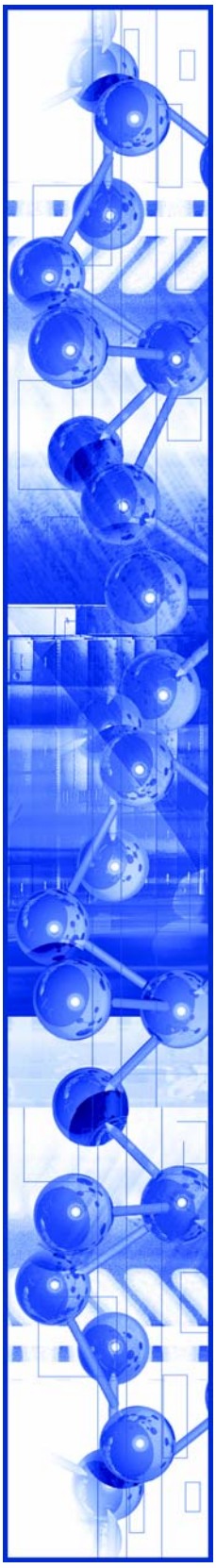


RiboZol™ Plus RNA Purification Kit

Code	Description	Size
N643-Kit	RiboZol™ Plus RNA Purification Kit *Includes*: 60mls RiboZol™ RNA Extraction Reagent (2 x 30ml) RiboZol™ Wash Buffer, 22mls (add 50ml 95% Ethanol for working stock) RiboZol™ Elution Buffer, 6ml RiboZol™ Spin Columns, 50 pieces RiboZol™ Collection Tubes, 50 pieces	50 preparations

General Information:

AMRESCO's RiboZol™ Plus RNA Purification Kit purifies and recovers all sizes and species of RNA, including large RNAs such as ribosomal RNA (rRNA) or messenger RNA (mRNA), and small RNAs less than 200 nucleotides including microRNAs (miRNA) and small inhibitory RNAs (siRNA). The kit combines the benefits of organic RNA extraction using RiboZol™ RNA Extraction Reagent with the ease and convenience of spin column technology. RiboZol™ RNA Extraction Reagent extracts total RNA from cells or tissue, including difficult sample types. Column chromatography employs a proprietary resin as the separation matrix to eliminate the time-consuming isopropanol precipitation step of the standard RiboZol™ RNA purification procedure. Up to 35 µg of total RNA may be concentrated per column. The RiboZol™ Plus RNA Purification Kit provides sufficient material for 50 total RNA purification procedures.

With the RiboZol™ Plus RNA Purification Kit biological samples are first disrupted directly into RiboZol™ RNA Extraction Reagent. Chloroform is added to the homogenate which is centrifuged to separate the three solution phases. RNA is recovered in the upper aqueous phase, which is diluted with ethanol and applied to the proprietary spin column. RNA binds to the column while contaminating proteins, DNA and nucleotides are removed during the washing step with the included Ribozol™ Washing Buffer. Purified RNA is then eluted with the included RiboZol™ Elution Buffer. Multiple purifications can be completed in less than 1 hour with ≥ 90% recovery.

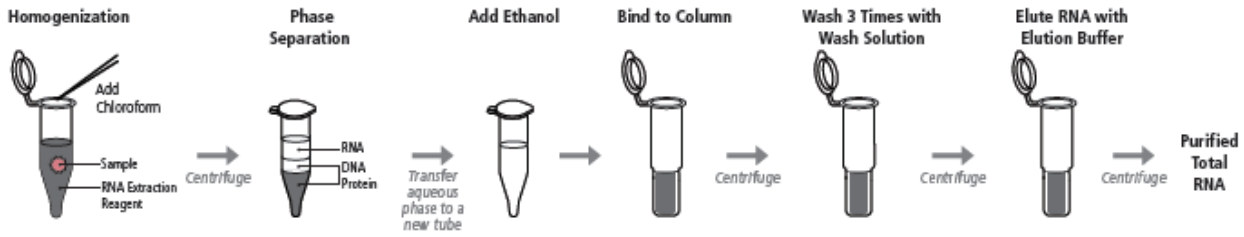
Total RNA purified with AMRESCO®'s RiboZol™ RNA Purification Kit is compatible with most downstream applications including Northern blot and dot blot analysis, RNAse protection assays, microarray analysis, molecular cloning and mRNA isolation. DNAse treatment is recommended for maximum removal of DNA prior to sensitive downstream applications such as quantitative RT/PCR.

Storage/Stability:

Product is stable for 1 year when stored tightly sealed at room temperature for 1 yr.

Application Disclaimer

For Research Use Only.
Not for Therapeutic or Diagnostic Use.



➥Note: **Hazard precaution. AMRESCO® RiboZol™ RNA Extraction Reagent contains phenol, which is a poison and can cause burns. Other ingredients are irritants. Protect skin and clothing. USE GLOVES AND EYE PROTECTION. Use a chemical fume hood in order to avoid breathing the vapor. Heed all warnings on the bottle and in the MSDS. In case of contact, immediately flush eyes or skin with a large amount of water for at least 15 minutes and seek immediate medical attention.**

➥Note: **General precautions working with RNA:** Although AMRESCO® RNA Extraction Reagent protects RNA from degradation by RNase activity, improper technique can introduce RNase at any point in the isolation procedure. Certain precautions should be taken when handling RNA:

1. Always wear disposable gloves when handling the sample in order to prevent contamination from mold and/or bacteria commonly found on the skin.
2. Sterile, disposable plasticware is recommended when working with RNA. In applications that require the use of non-disposable plasticware or glass, items must be treated for the removal of RNase. Glass items can be treated by baking them at 150°C for 4 hours. Plasticware or glassware can either be treated with RNase Inhibitor (E633), NucleasEliminator™ (E891) or soaked for 10 minutes in 0.5M NaOH, rinsed with water, and then autoclaved. Use designated RNA lab items, particularly automatic pipettes, to prevent cross-contamination from shared equipment.
3. NucleasEliminator™ Spray (E891-100ML-PUMP) or NucleasEliminator™ Wipes (E891-25PK) can be used to remove RNase contamination from large working surfaces.

Additional required materials not supplied:

Bench top centrifuge

Chloroform (0757)

Ethanol, 95%; in RNAse-free water.

Ethanol, 70%; in RNase-free water

RNAse-free microcentrifuge tubes to collect eluted RNA

PRIOR TO USE OF RIBOZOL™ PLUS RNA PURIFICATION KIT

- A variable speed centrifuge is recommended to obtain maximum yields of RNA.
- Ensure that all solutions are at room temperature prior to use.
- **Prepare a working concentration of RiboZol™ Wash Buffer**
  - Add 50ml of 95% ethanol (user supplied) to the supplied bottle containing concentrated RiboZol™ Wash Buffer. This will prepare a final volume of 72 ml.

RNA Isolation and Purification Procedure:

➥**Note:** Unless otherwise stated, the isolation procedure should be carried out at room temperature (15 to 30°C).

1) Sample Homogenization/Lysis:

- **Tissue:**
  - Using a glass-Teflon ® or power homogenizer, homogenize tissues in 1 ml of AMRESCO® RNA Extraction

Reagent per 50-100mg of tissue. It is important to ensure that the total tissue volume is not greater than 10% of the volume of AMRESCO® RNA Extraction Reagent.
    - Once the tissue has been completely homogenized, proceed to Step 2.
- **Adherent Cells:**
    - Cells grown in a monolayer can be lysed directly in the culture dish.
    - Using sterile technique, discard media and add 1mL of AMRESCO® RNA Extraction Reagent per 10cm^2 of culture dish area.
    - Lyse cells by passing them several times through the tip of a pipette.
    - Transfer cells to an RNase-free tube and proceed to Step 2.
- **Suspension Cells:**
    - Suspension cells should be pelleted by centrifugation in an RNase-free tube.
    - Following centrifugation, discard the supernatant and resuspend the pellet in 1mL of AMRESCO® RNA Extraction Reagent per 5-10^6 animal, plant or yeast cells or 1X10^7 bacterial cells. Avoid washing cells before the addition of AMRESCO® RNA Extraction Reagent as it tends to result in the degradation of mRNA.
    - Lyse cells by passing them several times through the tip of a pipette.
    - Proceed to Step 2.

➥**Note:** At this point, samples can be stored for at least 1 month at -60 to -70°C. Do not add chloroform prior to storage.

➥**Note:** Enzymatic digestion may be necessary for yeast or bacteria that are not easily compromised by mechanical shearing.

➥**Note:** Total cellular disruption is critical for high quality and yield of RNA. Disruption methods should be optimized to the sample. Various mechanical or enzymatic homogenization techniques may be used individually or in combination.

2) Separation of Phases:

- In order to ensure the complete dissociation of nucleoprotein complexes, incubate the homogenized sample for 5-10 minutes at room temperature.
- Add 200uL of chloroform per 1mL of AMRESCO® RNA Extraction Reagent and securely cap the tube.
- Shake the tube vigorously for 15 seconds to mix the sample, and then incubate the sample for 2-3 minutes at room temperature.
- Centrifuge the sample at 12,000 *x g* for 15 minutes at 4°C.
- Following centrifugation, three phases should be apparent: a lower red, phenol-chloroform phase, a white interphase and a colorless, upper, aqueous phase. RNA will be located exclusively in the upper aqueous phase.

➥**Note:** Do not attempt to remove the entire aqueous layer in the following step. To avoid contamination with protein, DNA, lipids and carbohydrates that often appear as debris or flocculent material at the interface, carefully remove only about 80% of the clear upper aqueous phase and if necessary use a back extraction to dilute and recover the remaining 10-20% of the original aqueous phase. Back extract the remaining aqueous phase adding an equal volume of RNA Extraction Reagent to the remaining phenol solution. Vortex the solution, centrifuge to separate the layers, and again remove the top aqueous layer as described above. Combine the two aqueous layers, and proceed to step 3, below.

3) Preparation of Sample:

- Transfer the aqueous phase to a new, RNase-free microcentrifuge tube.
- Precipitate the RNA by adding 1 volume of 70% ethanol (user-provided) to the aqueous phase in this new tube.
- Mix by vortexing for 10 seconds.

4) Binding to Column:

- Assemble a column with one of the provided collection tubes.
- Apply up to 600 µl of the RNA mixed with the ethanol from the previous step onto the column.
- Centrifuge for 1 minute at 14,000 x g.

- Discard the flowthrough, and reassemble the spin column with the collection tube.

Note: If the volume of RNA/ethanol obtained in step 3 exceeds 600 µl, add the mixture in steps not to exceed 600 µl each, until all the remaining RNA/ethanol has been applied to the column.

Note: If removal of genomic DNA contamination is critical, on-column DNase I treatment may be performed at this step. See Appendix A for instructions.

5) Column Wash:

- Apply 400 µl of RiboZol™ Wash Buffer to the column.
- Centrifuge for 1 minute at 14,000 x g.

Note: Make sure the entire wash solution has passed through into the collection tube by visually inspecting the column. If the entire wash volume has not passed through the column, spin for an additional minute.

1) Discard the flowthrough and reassemble the spin column with the collection tube.
2) Wash the spin column a second time by adding 400 µl of RiboZol™ Wash Buffer and centrifuging for 1 minute. Discard the flowthrough and reassemble the spin column with the collection tube.
3) Wash the spin column a third time by adding 400 µl of RiboZol™ Wash Buffer and centrifuging for 1 minute. Discard the flowthrough and reassemble the spin column with the collection tube.
4) Completely dry the resin by spinning the column 2 minutes.
5) Discard the collection tube.

5) RNA Elution:

- Place the column into an RNAse-free microcentrifuge tube (user provided).
- Add 50 µl of RiboZol™ Elution Buffer to the column.
- Centrifuge for 2 minutes at 2000 x g (2,000 rpm), followed by 1 minute at 14,000 x g (14,000 rpm).
- Elute the entire volume from the column. Note the volume eluted from the column. If the entire volume has not been eluted, spin the column for an additional minute at 14,000 x g.

Note: To obtain higher concentrations of RNA, a lower elution volume may be used but should be greater than 20µl of RiboZol™ Elution Buffer.

Note: A second elution may be performed into a separate microcentrifuge tube by repeating the RNA elution steps above.

6) RNA Storage:

- The eluted, purified RNA sample may be stored -20°C for a short period of time. Long term storage should be at -70 °C.

Appendix A: On-Column DNase treatment

For sensitive downstream applications, removal of any residual genomic DNA contamination may be performed with RNase-free DNase I directly on the RiboZol™ Plus Spin Column.

1) Prepare a 100 ul aliquot for each column of 0.25 Kunitz U/µl DNase I (RNase-free). Prepare the working stock according to the manufacturer's instructions, or dilute stock DNase I in reaction buffer to achieve a final concentration of 0.25 Kunitz/ µl.

   Reaction Buffer:
   40mM Tris pH 7.0
   10mM $MgCl_2$
   3mM $CaCl_2$

2) Perform total RNA Isolation procedure up to and including "Binding to Column" (Step 4).
3) Apply 400 µl of RiboZol™ Plus Wash Buffer to the column and centrifuge for 2 minutes. Discard the flowthrough and reassemble the spin column with its collection tube.
4) Apply 100 µl of the RNase-free DNase I solution prepared in Step 1 to the column and centrifuge at 14,000 x g (~14,000 rpm) for 1 minute.

Note: Make sure that the entire volume of solution passes through the column. If needed, spin an additional minute at 14,000 x g.

5) After centrifugation, pipette the flowthrough that is present in the collection tube back onto the spin column. This step ensures that maximal

DNase I activity is achieved and maximum yields of RNA is obtained.

6) Incubate the assembled column at 25-30°C for 15 minutes.

7) After incubation proceed directly to Step 5 "Column Wash". Do not centrifuge column prior to adding RiboZol™ Wash Buffer.

Appendix B: Troubleshooting

Symptom: Poor RNA Recovery

1) Column may be clogged.
   a. Make sure than no more than 35 µg of RNA is used as the input per column.
2) Wrong elution solution applied to column.
   a. The elution buffer provided in the kit is recommended for maximum RNA recovery.
3) Ethanol omitted from lysates.
   a. The appropriate amount of ethanol must be added to the aqueous phase solution containing RNA to ensure binding to the column.
4) Ethanol omitted from the RiboZol™ Plus Wash Buffer.
   a. The correct amount of 95% ethanol (50ml) must be added to the Wash Buffer bottle prior to use.

Symptom: Clogged Column

1) Input RNA too high.
   a. Make sure than no more than 35 µg of RNA is used as the input per column
2) High amounts of genomic DNA in the sample.
   a. Pass the lysate through a 25 gauge needle attached to a syringe several times to shear the genomic DNA prior to loading onto the RiboZol™ Plus Spin Column.
3) Temperature of the centrifuge spins too low.
   a. The centrifuge temperature should remain at room temperature throughout the procedure. Low temperature may cause precipitation and clog the columns.

Symptom: Degraded RNA

1) RNase contamination.
   a. RNases may be introduced at any stage during the procedure. Make sure you have followed the recommended precautions while working with RNA.
2) Procedure time too slow.
   a. The procedure must be performed quickly to preserve RNA integrity.
3) Improper storage of the purified RNA.
   a. Store the eluted RNA at -20°C only for short term. Samples should be maintained at -70°C for long-term storage.

Symptom: DNA or Genomic DNA Contamination

1) Too much starting material used.

a) Significant amount of genomic DNA contaminate preparations if too much starting material is used. Follow recommendations in Step 1 - Sample Homogenization/Lysis. In addition, DNAse digestion may be performed on the column as described in Appendix A.

Related Products

Code	Product
N580-100ml N580-200ml N580-30ml	RiboZol™ RNA Extraction Reagent
0757-950ml	Chloroform
0918-1L 0918-4L	Isopropyl Alcohol
0606	Formamide
0493	Formaldehyde
E174-5G E174-25G	Diethylpyrocarbonate (DEPC)
E891-100ml E891-500ml	NucleasEliminator™
E891-50ml-PUMP E891-100ml-PUMP	NucleasEliminator™ Spray
0710-500G	Agarose I™, 500 g General Use (also available as tablets, K857-100TABS)
Buffers	
0670	MOPS buffer
N633-50ml N633-12x1ml	RiboReserve™ RNA Storage Buffer
N634-50ml N634-12x1ml	TE, pH 7.0
N632-2x0.5ml	Glycogen Solution, 20 mg/ml
Markers	
N603-Kit	RiboReady ™100 b RNA Ladder 8 bands ranging from 100-1000 nts
N604-Kit	RiboReady ™ 1K b RNA Ladder 9 bands ranging from 200 to 4000 nts

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