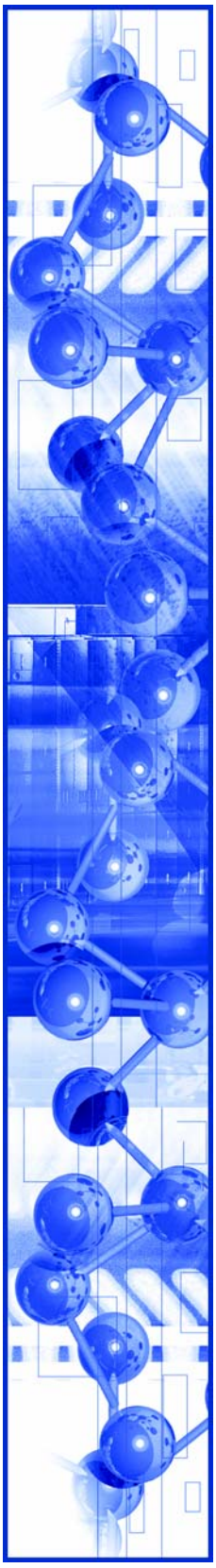


RiboZol™ RNA Extraction Reagent

Code	Description	Size
N580-100ML	RiboZol™ RNA Extraction Reagent	100 ml
N580-200ML		200 ml
N580-Q-30ML		30 ml

General Information:

AMRESCO's Ribozol™ RNA Extraction Reagent is a single phase phenol solution that is used for the isolation of total RNA from a variety of cell and tissue types. Homogenization or disruption directly in Ribozol™ RNA Extraction Reagent directly inhibits RNase activity to substantially minimize degradation of all classes of RNA. The simple and effective procedure for isolation in Ribozol™ RNA Extraction Reagent includes homogenization, phase separation, RNA precipitation, RNA wash and solubilization.

Total RNA isolated with Ribozol™ RNA Extraction Reagent can be used for many downstream applications including:

- Northern blot and dot blot analysis
- RNAse protection assays
- Molecular cloning
- mRNA isolation
- RT/PCR - In some situations, DNAse treatment is recommended prior to PCR

Storage/Stability:

Store product at 4^{o} C.

Application Disclaimer

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

RNA Isolation Procedure:

Supplied Materials:
Ribozol™ RNA Extraction Reagent

Additional required materials not supplied:
Chloroform (Code #: 0757)
Isopropanol (Code #: 0918)
Ethanol, 75% in RNase-free water
RNase-free water (Code #: E476-500ML) or a 0.5% SDS solution (SDS solution must be prepared using RNase-free water).

Note: Hazard precaution. 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 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 Ribozol™ 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 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 nondisposable 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 can either be treated with RNase Inhibitor (E633) or soaked for 10 minutes in 0.5M NaOH, rinsed with water, and then autoclaved.
3. Use designated RNA lab items, particularly automatic pipettes, to prevent cross-contamination from shared equipment.
4. NucleasEliminator™ Spray (E891-100ML-PUMP) or NucleasEliminator™ Wipes (E891-25PK) can be used to remove RNase contamination from large working surfaces.

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

1) Sample Homogenization/Lysis:

a. Tissue:
- Using a glass-Teflon® or power homogenizer, homogenize tissues in 1 ml of Ribozol™ RNA Extraction Reagent per 50-100 mg of tissue. It is important to ensure that the total tissue volume is not greater than 10% of the volume of Ribozol™ RNA Extraction Reagent.
- Once the tissue has been completely homogenized proceed to Step 2.

b. Adherent Cells:
- Cells grown in a monolayer can be lysed directly in the culture dish.
- Using sterile technique, discard media and add 1 mL of Ribozol™ RNA Extraction Reagent per 10 cm^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.

c. Suspension Cells:
- Suspension cells should be pelleted by centrifugation in an RNase-free tube.
- Following centrifugation, discard the supernatant and resuspend the pellet in 1 mL of Ribozol™ RNA Extraction Reagent per $5x10^6$ animal, plant or yeast cells or $1X10^7$ bacterial cells. Avoid washing cells before the addition of Ribozol™ 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 1 mL of Ribozol™ RNA Extraction Reagent added in step 1 and tightly secure 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. a lower red, phenol-chloroform phase
  b. a white interphase
  c. a colorless, upper, aqueous phase.
- RNA will be located exclusively in the upper aqueous phase. Carefully remove only about 80% of the clear upper aqueous phase. Do not attempt to remove the entire aqueous layer to avoid contamination with protein, DNA, lipids and carbohydrates that appear as debris or flocculent material at the interface.
- Back extract to recover the remaining 10-20% of the original aqueous phase by adding an equal volume of Ribozol™ RNA Extraction Reagent to the remaining phenol solution.
- Vortex the solution and centrifuge to separate the layers.
- Remove the top aqueous layer as described above.
- Combine the two aqueous layers and proceed to step 3.

3) Precipitation of RNA:

- Transfer the aqueous phase to a new, RNase-free tube and precipitate the RNA by adding 0.5 mL of isopropanol per 1 mL of Ribozol™ RNA Extraction Reagent used in the initial homogenization.
- Incubate samples for 10 minutes at room temperature and then centrifuge at 12,000 *x g* for 10 minutes at 4°C.
- A white or gel-like pellet of precipitated RNA should form along the side and bottom of the tube. The size of the pellet will depend on the amount of cell/tissue starting material. A pellet of very pure RNA may be nearly transparent and difficult to see.

4) Washing:

- Carefully remove the supernatant without disrupting the RNA pellet.
- Wash the pellet at least once with 75% ethanol prepared with RNase-free water.
- For each wash add 1 mL of ethanol per 1 mL of Ribozol™ RNA Extraction Reagent used in the initial homogenization, vortex, and centrifuge at 7,500 *x g* for 5 minutes at 4°C.

Note: Prior to centrifugation, the RNA precipitate can be stored in 75% ethanol either at 4°C for one week or at -20°C for one year.

5) Re-dissolve the RNA pellet:

- Following the final ethanol wash, carefully remove the ethanol without disrupting the pellet.
- Briefly air-dry the pellet for 5-10 minutes. Do not dry the pellet completely as it decreases the solubility of the RNA.
- Dissolve RNA in RNase-free water, 0.5% SDS solution or other RNA storage solutions. Use 50 uL for every $5x10^6$ cells or 10 cm^2 dish (SDS is not recommended when RNA is to be used in downstream enzymatic reactions).
- Pass the pellet several times through a pipette tip, and incubate for 10 minutes at 55 to 60°C to completely dissolve.

6) Determination of RNA yields and purity

- RNA concentration be determined by absorbance at A_{260}:

RNA Concentration = A_{260}/(l x e)
l = cuvette path length (cm)
e = RNA extinction coefficient (25ul/ug/cm)

Expected yield of RNA from 10 x 10^6 cultured cells is 150-200 ug.

RNA purity is determined by the ratio of absorbance at A_{260}/A_{280}. High quality RNA should be between 1.6 and 1.8 but may vary depending on the resuspension solution and the RNA source.

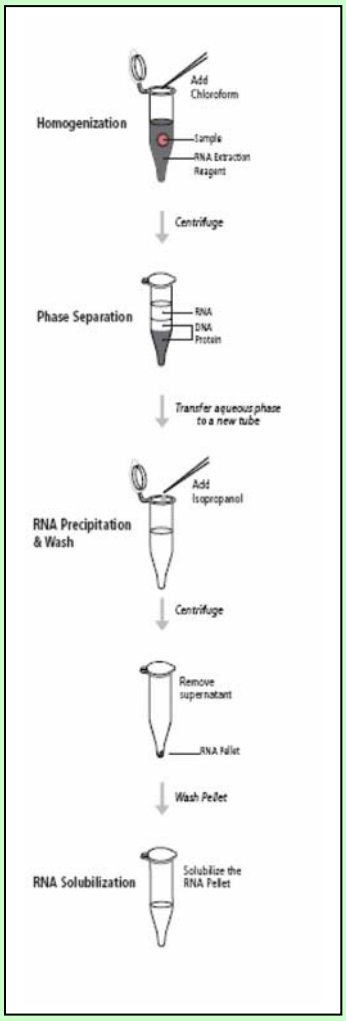
Note: Water or solution used for RNA solubilization should be made RNAse free by diethyl pyrocarbonate (DEPC) treatment.

Related Products :

Code	Product
0757	Chloroform
0918	Isopropanol
E476-500ML	RNase-free water (DEPC)

Corporate Headquarters
AMRESCO Inc.
30175 Solon Industrial Pkwy.
Solon, OH 44139 USA
Tel: (440) 349-1199
Toll free: 1-800-829-2805 (US & Canada)
Fax: (440) 349-2441
e-mail: info@amresco-inc.com

Ribozol™ RNA Extraction Reagent Rapid Purification Procedure



www.interchim.com
interchim

211 bis Avenue Kennedy - BP 1140
03103 Montluçon - France
33 (0) 4 70 03 88 55
Fax 33 (0) 4 70 03 82 60
e-mail interchim@interchim.com

Agence Paris - Normandie
33 (0) 1 41 32 34 40
Fax 33 (0) 1 47 91 23 90
e-mail interchim.paris@interchim.com