# RiboZol™ RNA Extraction Reagent

| Code | Description | Size |
|---|---|---|
| N580-30ML-SAMPLE | **Ribozol™ RNA Extraction Reagent** | 30 mL |
| N580-30ML | **Ribozol™ RNA Extraction Reagent** | 30 mL |
| N580-100ML | **Ribozol™ RNA Extraction Reagent** | 100 mL |
| N580-200ML | **Ribozol™ RNA Extraction Reagent** | 200 mL |

## General Information

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.

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 (DNase treatment may be required)

## Storage/Stability

Store at room temperature (18 – 26°C). RiboZol™ is stable for 2 years.

## Product Use Limitations

For research use only. Not for therapeutic or diagnostic use.

# Directions for Use

## Materials Supplied

RiboZol™ RNA Extraction Reagent

## Required Materials Not Supplied

| RNA Isolation | DNA Isolation | Protein Isolation |
|---|---|---|
| Chloroform | Ethanol | Ethanol |
| Isopropyl alcohol | 0.1 M sodium citrate, 10% ethanol | Isopropyl alcohol |
| Ethanol, 70% | Ethanol, 75% | 0.3 M guanidine HCl in 95% ethanol |
| Nuclease-free water | 8 mM NaOH | 1% SDS |

## Protocol/Procedure:

**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.**

**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 plastic ware is recommended when working with RNA. In applications that require the use of non-disposable plastic ware 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. Plastic ware can either be treated with RNase Inhibitor or soaked for 10 minutes in 0.5 M 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™ (E891) can be used to remove RNase contamination from large working surfaces.

## RNA Isolation

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

**1) Sample Homogenization/Lysis:**

**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.

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

**Note**: Instructions for homogenization of biological liquids are included in the "Special Instructions for Biological Fluids" following the standard procedure. See page 7.

**a. Tissue:**

- Using a glass-Teflon ® or power homogenizer, homogenize tissues in 1 mL of RiboZol™ 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™.
- Once the tissue has been completely homogenized proceed to Step 2.

**b. Plant Tissue:**

**Note:** Disruption methods should be optimized based on the fibrous nature of the plant and the types and levels of polysaccharides. Various mechanical or enzymatic homogenization techniques may be used individually or in combination to ensure complete cellular disruption.

- Using a glass-Teflon ® or power homogenizer, homogenize tissues in 1 mL of RiboZol™ 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™.
- Centrifuge homogenate at 12,000 *x g* for 10 minutes at 4 °C to remove insoluble material including extracellular membranes and polysaccharides.
- Transfer the clear supernatant to a fresh tube and proceed to phase separation, Step 2.

**c. 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™ 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.

**d. Suspended Cells:**

- Suspension cells should be pelleted by centrifugation in an RNase-free tube.
- Following centrifugation, discard the supernatant and re-suspend the pellet in1 mL of RiboZol™ per $5x10^6$ animal, plant or yeast cells or $1X10^7$ bacterial cells. Avoid

washing cells before the addition of RiboZol™ 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.

**e. Biological Fluids** (including samples of human, animal, plant, yeast, bacterial and viral origin)**:** See special instructions for biological fluids at the end of the standard procedure.

**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.

**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 200 µL of chloroform per 1 mL of Ribozol™ 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.
- Re-extract to recover the remaining 10-20% of the original aqueous phase by adding an equal volume of nuclease-free water or other appropriate buffer 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. **Note:** This will increase overall yield of RNA, but the final RNA concentration will be more dilute.

**Note:** Save interphase for DNA Isolation procedure to recover DNA from sample.

**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™ 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.

**Precipitation of RNA from Plant Tissue**

- Transfer the aqueous phase to a new, RNase-free tube.
- For each 1 mL of Ribozol™ used for homogenization, add 0.25 mL of isopropanol followed by 0.25 mL of 0.8M sodium citrate/1.2M NaCl.
- Mix solution and store for 5 – 10 minutes at room temperature.
- Centrifuge at 12,000 *x g* for 8 minutes at 4-25°C.
- Proceed to Washing, step 4.

**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 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 µL 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 can be determined by absorbance at A260:
RNA Concentration = A260/(l x e)
l = cuvette path length (cm)
e = RNA extinction coefficient (25 µL/µg/cm)
Expected yield of RNA from 10 x $10^6$ cultured cells is 150-200 µg. RNA purity is determined by the ratio of absorbance at A260/A280. High quality RNA should be between 1.6 and 1.8, but may vary depending on the re-suspension solution and the RNA source.

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

## Procedure-DNA Isolation

**1) Precipitation of DNA:**

- Ensure complete removal of the aqueous phase obtained in RNA Isolation step 2.

- Add 0.3 mL 100% ethanol per mL of RiboZol™ reagent used for homogenization to the interphase/organic phase and mix by inversion.
- Incubate 3 minutes at 15 - 30°C.
- Centrifuge at 2,000 *x g* for 5 minutes at 2 - 8°C.
- Discard or transfer supernatant to fresh tube. Save the phenol/ethanol supernatant in a new tube for protein isolation.

**2) Washing:**

- Wash DNA by adding 0.1 M sodium citrate/10% ethanol (1mL per every 1 mL RiboZol™ reagent used) to the pellet.
- Incubate 30 minutes at 15 – 30°C with intermittent mixing.
- Centrifuge at 2,000 *x g* for 5 minutes at 2 - 8°C.
- Repeat wash steps.
- Re-suspend DNA in 75% ethanol (1 mL per every 1 mL RiboZol™ reagent used).
- Incubate 10 – 20 minutes at 15 – 30°C with intermittent mixing.
- Centrifuge at 2,000 *x g* for 5 minutes at 2 - 8°C.

**3) Re-dissolve the DNA Pellet:**

- Briefly air-dry the pellet for 5-10 minutes. Do not dry the pellet completely as it decreases the solubility of the DNA.
- Dissolve DNA in 8 mM NaOH (~ pH 9).
- Centrifuge at > 12,000 *x g* for 10 minutes, if needed, to remove insoluble material.
- Adjust the pH with TE to pH 7 – 8.
- Store DNA at 4°C or -20°C.

## Procedure-Protein Isolation

**1) Precipitation of Protein:**

- To the phenol/ethanol supernatant saved in step 2 of the DNA Isolation procedure, add isopropyl alcohol (1.5 mL per mL RiboZol™ reagent used).
- Incubate 10 minutes at 15 – 30°C.
- Centrifuge at 12,000 *x g* for 10 minutes at 2 -8°C.
- Discard the supernatant.

**2) Washing:**

- Wash protein with 0.3 M guanidine hydrochloride in 95% ethanol (2 mL per every 1 mL RiboZol™ reagent used).
- Incubate 20 minutes at 15 – 30°C.
- Centrifuge at 7,500 *x g* for 5 minutes at 2 - 8°C.
- Repeat wash steps twice more.

**3) Re-dissolve the Protein Pellet**

- Dry the pellet by vacuum for 5 – 10 minutes.
- Re-suspend pellet in 1% SDS by pipetting up and down.
- Centrifuge at 10,000 *x g* for 10 minutes at 2 – 8°C to remove insoluble material, if necessary.
- Transfer supernatant containing protein to a fresh tube.
- Store protein at -20°C

**Special Instructions for Biological Fluids**

**1. Homogenization of Biological Fluids (including samples of human, animal, plant, yeast, bacterial and viral origin):**

- If sample volume is less than 0.25 mL, adjust volume to 0.25 mL with RNase-free water.
- Combine 0.75 mL of Ribozol™ with 0.25 mL of sample and lyse cells by passing the suspension through a pipette several times. Use at least 0.75 mL of Ribozol™ per 5-10 x $10^6$ cells.
- The volume ratio of Ribozol™ to sample should be 3:1.
- Proceed to Step 2

**2. Phase Separation for Biological Fluids:**

- Incubate samples for 5 minutes at room temperature.
- Add 200 µL of chloroform per 0.75 mL of Ribozol™.
- Cover and shake vigorously for 15 sec
- Incubate at room temperature for 2-15 minutes.
- Centrifuge at 12,000 *x g* at 4°C for 15 minutes.
- Following centrifugation, three phases should be apparent:
  a. a lower red, phenol-chloroform phase
  b. a white interphase
  c. a colorless, upper, aqueous

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.

**3. RNA Precipitation for Biological Fluids:**

- Transfer the aqueous phase to a new, RNase-free tube and precipitate the RNA by adding 0.5 mL of isopropanol per 0.75 mL of Ribozol™ used in the initial homogenization.
- Incubate samples for 5-10 minutes at room temperature.
- Centrifuge at 12,000 *x g* for 8 minutes at 4 – 25 °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. RNA Wash for Biological Fluids:**

- Decant supernatant without disturbing the RNA pellet.
- Add a minimum of 1 mL of 75% ethanol per 0.75 mL of Ribozol™ used in the initial homogenization.
- Vortex and centrifuge at 7,500 *x g* for 5 minutes at 25°C.
- Decant supernatant without disturbing the RNA pellet.
- Air dry for 3 – 5 minutes but do not let pellet dry completely. Complete drying reduces solubilization of RNA.

**5. RNA Solubilization for Biological Fluids:**

- Dissolve RNA in RNase-free water, 0.5% SDS solution or other RNA storage solutions. Use 50 µL 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.

## Frequently Asked Questions

| Questions | Answers |
|---|---|
| Why does my aqueous layer appear pink? | Not enough RiboZol ™ was added to the sample. Re-extract the aqueous layer with RiboZol ™ and chloroform. |
| Why is my yield so low? | 1. Samples were not immediately processed after cell or tissue collection.<br>2. RNase contamination during the procedure degraded the RNA.<br>3. Samples were not completely homogenized or lysed.<br>4. Final pellet not re-suspended completely. |
| Why is the $A_{260/280}$ ratio so low? | 1. Incomplete removal of organic phase.<br>2. Sample was homogenized in an insufficient amount of RiboZol™. |

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|---|---|
| Why do I have so much DNA contamination in my RNA sample? | 1. Too little RiboZol™ reagent used.<br>2. Organic solvents were present in sample source. |


## For Technical Support

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