PRODUCT: **RNAzol® RT** August 2023
Catalog No: RN 190
Storage: Store at room temperature

PRODUCT DESCRIPTION

RNAzol® RT is the most effective reagent for isolation of total RNA and small RNA from samples of human, animal, plant, bacterial and viral origin. This patented reagent (1) provides higher yield and quality of isolated RNA than previous reagents based on the single-step method (2, 3). RNAzol® RT isolates pure and undegraded RNA that is ready for RT-PCR without DNAse treatment.

RNAzol® RT separates RNA from other molecules in a single-step based on the interaction of phenol and guanidine with cellular components. No chloroform-induced phase separation is necessary to obtain pure RNA. A biological sample is homogenized or lysed in RNAzol® RT. DNA, proteins, polysaccharides and other molecules are precipitated from the homogenate/lysate by the addition of water and removed by centrifugation. The pure RNA is isolated from the resulting supernatant by alcohol precipitation, followed by washing and solubilization.

- The isolation procedure can be completed in less than one hour. The isolated RNA is ready for use in RT-PCR, qRT-PCR, microarrays, poly A+ selection, northern blotting, RNase protection assay and other molecular biology applications.
- RNAzol® RT isolates large RNA and small RNA in separate fractions. Alternatively, total RNA containing all classes of RNA in a single fraction can be isolated. In addition, RNAzol® RT allows for the sequential isolation of RNA and DNA.
- The RNAzol® RT procedure is performed at room temperature, including centrifugation.

STABILITY/STORAGE: RNAzol® RT is stable when stored at room temperature for at least two years from date of purchase.

SPECIAL HANDLING PRECAUTIONS.

RNAzol® RT contains phenol (corrosive liquid/poison) and guanidine thiocyanate (irritant). CAUSES BURNS. Can be fatal. When working with RNAzol® RT, use gloves and eye protection (face shield, safety goggles). Do not get on skin or clothing. Avoid breathing fumes. Read the warning note on the container and SDS. **In case of contact:** Immediately flush eyes or skin with a large amount of water for at least 15 minutes and if necessary seek medical attention.

I. PROTOCOLS FOR RNA ISOLATION.

There are two protocols for RNA isolation using RNAzol® RT. For fast and reliable isolation of RNA for gene expression studies, we recommend isolating the large RNA fraction according to the first protocol.

I-1. Isolation of Large RNA and Small RNA Fractions - Abbreviated Protocol

This protocol yields two RNA fractions; large RNA > 150 - 200 bases and small RNA < 150 - 200 bases.

Step	Procedure
1. Homogenization	- 1 ml RNAzol® RT + up to 100 mg tissue or 10^7 cells.
2. DNA/protein precipitation	- homogenate + 0.4 ml water, wait 5 - 15 min, 12,000 g x 15 min.
3. Large RNA precipitation	- 1 ml supernatant + 0.4 ml of 75% ethanol, wait 10 min, 12,000 g x 8 min.
4. Large RNA washes	- 0.4 ml 75% ethanol, 8,000 g x 1 - 3 min; wash twice.
5. Large RNA solubilization	- water or FORMAzol®.
6. Small RNA precipitation	- post-large RNA supernatant (Step 3) + 0.8 vol. isopropanol, wait 30 min, 12,000 g x 15 min.
7. Small RNA washes	- 0.4 ml 70% isopropanol, 8,000 g x 3 min, wash twice.
8. Small RNA solubilization	- water or FORMAzol®.

Due to lack of impurities in the large RNA fraction, the protocol yields RNA pellets that are significantly smaller than pellets obtained from samples processed with previous single-step reagents. The use of large RNA separated from small RNA eliminates possible interference between these two classes of RNA during an assay.

I-2. Isolation of Total RNA - Abbreviated Protocol

This protocol yields total RNA comprising all classes of RNA: large nuclear RNA, rRNA, mRNA, small RNA and microRNA down to 10 bases.

Step	Procedure
1. Homogenization	- 1 ml RNAzol® RT + up to 100 mg tissue or 10^7 cells.
2. DNA/protein precipitation	- homogenate + 0.4 ml water, wait 5 - 15 min, 12,000 g x 15 min.
3. BAN purification (optional)	- 1 ml supernatant + 5 µl 4-bromoanisole, wait 3 - 4 min, 12,000 g x 10 min.
4. RNA precipitation	- supernatant + 1 volume isopropanol, wait 15 min, 12,000 g x 10 min.
5. RNA washes	- 0.4 ml 75% ethanol, 4,000 g x 1 - 3 min; wash twice.
6. RNA solubilization	- water or Formazol®.

An optional purification step using 4-bromoanisole (BAN) can be used to further eliminate DNA contamination.

II. NOTES TO THE PROTOCOLS.

1. The rapid removal and immediate homogenization of tissue is critical for the integrity and yield of RNA. The most effective method is homogenization for 2 - 3 min in a Polytron-type homogenizer set at a high-speed.
- For tissues with high RNase activity such as pancreas, perform homogenization in cold RNAzol® RT. The resulting homogenate can be processed at room temperature.
- Brain samples require the use of a glass-Teflon homogenizer because of excessive foaming.

2. To estimate tissue weight used for the homogenization, place 1 - 5 ml of the reagent in a tube on a balance and tare it. Drop fresh or frozen tissue into the reagent, record the tissue weight and immediately homogenize the sample. After dispersing the tissue, supplement the homogenate with reagent to achieve the desired mg of tissue per ml of the reagent.
3. The RNA isolation can be interrupted and samples can be stored as indicated here:
- The sample homogenate, before addition of water, can be stored overnight at 4 C or for at least two years at -70 C. To thaw the sample, incubate at 37 - 40 C for 5 minutes with periodic mixing.
- The RNA precipitate can be stored in 75% ethanol overnight at room temperature (RNA Wash), for at least one week at 4 C, or at least one year at -20 C.

III. RNA ISOLATION TROUBLESHOOTING GUIDE.

Low yield. a) incomplete homogenization or lysis of samples; b) incomplete solubilization of the final RNA pellet.
260/280 ratio<1.6. a) insufficient volume of the reagent was used for homogenization; b) acidic water was used for the OD measurement; c) incomplete solubilization of the RNA pellet; d) proteoglycan or polysaccharide contamination.
RNA degradation. a) tissues were not immediately processed or frozen in liquid nitrogen after removal from an animal; b) samples used for RNA isolation were stored at -20 C instead of at -70 C; c) cells were dispersed by trypsin digestion; d) aqueous solutions or tubes used for solubilization of RNA were not RNase-free.
DNA contamination. a) too much tissue or cells were used for the volume of the reagent used for homogenization; b) samples used for RNA isolation contained organic solvents, strong buffers, salt or alkaline solution.
The following modifications of the DNA precipitation (Step 2) will further reduce DNA contamination:
- After addition of water (Step 2), extend the incubation time to 15 minutes before sedimentation.
- After addition of water, sediment the precipitated DNA (Step 2) at 16,000 g.

Removal of proteoglycan and polysaccharide contamination. After homogenization (Step 1), perform an optional additional centrifugation at 12,000 g for 5 min to clarify the initial crude homogenate. Transfer the clear supernatant to a clean tube and add 0.4 volumes of water, shake vigorously and continue with the RNA Isolation Protocol as described in Step 2.
Removal of fat. After homogenization (Step 1), centrifuge samples with a high fat content at 12,000 g for 5 min. Excess lipid forms a layer at the top of the tube. Remove most of this layer with a pipette or syringe. Mix the remaining homogenate with 0.4 volumes of water, shake vigorously and continue with the RNA Isolation Protocol as described in Step 2.

IV. RECOVERY OF DNA.

For use in PCR, DNA can be recovered from the DNA-containing pellet obtained after centrifugation of the homogenate/lysate (Step 2), by using either DNAzol® or DNAzol® Direct.

DNAzol®. Remove any residual supernatant without disturbing the pellet. Vigorously mix the pellet with 8 - 10 volumes of DNAzol® (MRC, DN 127) and perform DNA isolation as described in the DNAzol® brochure.

DNAzol®Direct. Remove any residual supernatant without disturbing the pellet. Vigorously mix the pellet with a minimum of 10 volumes of DNAzol®Direct. Take an aliquot of the resulting mix, dilute it with water and use it for PCR. Heating the DNAzol® Direct - pellet mixture for 10 min at 60 C improves DNA recovery.

REFERENCES.

1. Chomczynski P, Reagents and methods for isolation of purified RNA, US Patent # 7,794,932. Reagents for isolation of purified RNA, US Patent # 8,367,817. International Patents.
2. Chomczynski P, and Sacchi N (1987) Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162, 156 - 159.
3. Chomczynski P (1993) A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. Biotechniques 15, 532 - 537.
4. Wilfinger W, Mackey K and Chomczynski P (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. Biotechniques 22, 474 - 481.

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RNAzol® RT
PROTOCOL FOR ISOLATION OF LARGE RNA AND SMALL RNA FRACTIONS

This protocol yields a large RNA fraction containing RNA > 150 - 200 bases, and a small RNA fraction containing RNA < 150 - 200 bases. Due to multiple splicing sites, some small RNA can be detected in both the large and small RNA fractions.

Reagents required but not supplied: ethanol, isopropanol and RNase-free water. We recommend the use of disposable polypropylene tubes (see MRC catalog). Tubes from other sources should be tested to ensure integrity during centrifugation at 12,000 g with RNAzol® RT.

The isolation is performed at room temperature, centrifugation at 4 - 28 C, and precipitation of small RNA at 4 - 28 C.

1. HOMOGENIZATION.

A. Tissues. Homogenize a tissue sample in a Polytron-type homogenizer or glass-teflon homogenizer using up to 100 mg of tissue per 1 ml of RNAzol® RT. When processing tissues with high DNA content such as spleen, use 50 mg of tissue per 1 ml reagent.

For clarity of presentation, the protocol describes isolation using 1 ml of RNAzol® RT. For the most practical/economical processing of samples, use 1.5 - 2 ml microcentrifuge tubes. For example, use a 0.88 ml aliquot of the homogenate (0.88 ml = 0.8 ml reagent + 80 mg tissue) and freeze the rest of the homogenate for later use. The homogenate can be stored at -20 or -70 C for at least two years.

B. Cells. Cells grown in monolayer should be lysed in the culture dish by addition of RNAzol® RT. Remove culture medium and add at least 1 ml of reagent per 3.5 cm culture dish (10 cm^2). Pass the lysate through a pipette several times to ensure lysis. Cells grown in suspension should be sedimented first and then lysed by the addition of RNAzol® RT. Add at least 1 ml of RNAzol® RT per 10^7 cells and lyse cells by repeated pipetting.

Washing cells before the addition of RNAzol® RT is not recommended as it may contribute to RNA degradation.

For cells grown in monolayer, use the amount of RNAzol® RT based on the area of the culture dish and not on cell number. An insufficient amount of RNAzol® RT will result in DNA contamination of the isolated RNA.

C. Liquid Samples. Homogenize/lyse liquid samples using 1 ml of RNAzol® RT per 0.4 ml of a liquid sample. For processing a small volume sample, mix the sample with 1 ml of RNAzol® RT and supplement the mixture with water to approach the sample + water volume of 0.4 ml.

D. Samples With High Fat Content. After complete homogenization, centrifuge high-fat samples at 12,000 g for 5 minutes. Excess lipid forms a layer at the top of the tube. Remove most of this layer with a pipette or syringe. Mix the remaining homogenate with 0.4 volumes of water, shake vigorously and continue RNA isolation as described in Step 2.

2. DNA, PROTEIN AND POLYSACCHARIDE PRECIPITATION.

Add to the homogenate/lysate 0.4 ml of water per 1 ml of RNAzol® RT used for homogenization. Shake the resulting mixture vigorously for 15 seconds and store for 5 - 15 minutes. Samples with 100 mg tissue/ml RNAzol® RT require a 15 minute storage at room temperature. Centrifuge the sample at 12,000 g for 15 minutes. Following centrifugation, DNA, proteins and most polysaccharides form a semisolid pellet at the bottom of the tube. The RNA remains soluble in the supernatant.

Centrifugation at this and other steps of the protocol can be performed at 4 - 28 C.

The DNA-protein pellet in a sample with 100 mg tissue/ml reagent constitutes about 10% of the total volume of the homogenate-water mixture (for 80 mg/ml it is about 8%).

3A. PRECIPITATION OF LARGE RNA FRACTION.

Transfer 1 ml of the supernatant (75% of total supernatant volume) to a new tube, leaving a layer of the supernatant above the DNA/protein pellet. Precipitate RNA by mixing the transferred 1 ml of supernatant with 0.4 ml of 75% ethanol (v/v). Store sample for 10 minutes and centrifuge at 12,000 g for 8 minutes. RNA precipitate forms a white pellet at the bottom of the tube. Transfer the supernatant to a new tube and store it at 4 C or at -20 C for isolation of small RNA.

It is safe to collect up to 85% of the supernatant.

The large RNA fraction contains RNA > 150 - 200 bases including large nuclear RNA, all mRNA and rRNA.

The supernatant containing small RNA can be stored at -20 C for at least one year.

The precipitation of RNA at this step is so selective that a special carrier (Precipitation Carrier, PC173) was designed for isolation of a small amount of RNA (< 10 µg). Add Precipitation Carrier to the initial homogenate or to the supernatant and mix it just before precipitation of the large RNA fraction. Glycogen and MRC Polyacryl Carrier do not precipitate at this step.

3B. PRECIPITATION OF SMALL RNA FRACTION.
Mix 0.8 volumes of isopropanol with the supernatant obtained after precipitation of large RNA in Step 3A. Store the sample for 30 minutes at room temperature or 4 C and sediment the precipitated RNA at 12,000 g for 15 minutes at 4 - 28 C. The RNA precipitate forms a white pellet at the bottom of a tube. The sample may be stored at 4 C to potentially increase yield of some microRNAs.
The small RNA fraction contains RNA < 150 - 200 bases including small rRNA, tRNA and microRNA down to 10 bases.
To improve the handling of small amounts of RNA(< 3 µg), mix 1 - 2µl of Polyacryl Carrier (PC152) with the supernatant and proceed with small RNA precipitation using isopropanol as described above.

4. RNA WASHES. Wash the RNA twice by mixing the large RNA pellet (Step 3A) with 75% ethanol (v/v) or the small RNA pellet (Step 3B) with 70% isopropanol (v/v). Centrifuge the pellets at 4,000 - 8,000 g for 1 - 3 minutes. When performing the isolation in 1.5 ml microcentrifuge tubes, use 0.4 - 0.6 ml of the alcohol solution. For samples processed in larger tubes use 0.5 ml of alcohol solution per 1 ml of the supernatant used for precipitation. Remove the alcohol solution using a micropipette.

5. RNA SOLUBILIZATION. Dissolve the RNA pellet, without drying, in water or FORMAzol® (MRC, FO 121) to approach an RNA concentration of 1 - 2 µg/µl for the large RNA fraction and about 0.1 µg/µl for the small RNA fraction. For solubilization in water, vortex the RNA pellet at room temperature for 2 - 5 minutes. Tubes and water used for the RNA solubilization should be RNase-free. Solubilization in FORMAzol® requires incubation of RNA for 10 - 20 minutes followed by vortexing or repetitive pipetting.
Drying the RNA pellet is not recommended as this greatly decreases its solubility.
Solubilization of RNA in FORMAzol® can be facilitated by incubation at 55 C for 5 - 15 minutes.
Samples dissolved in FORMAzol® should be diluted at least 5 fold with buffer before application on RNA Chips (Agilent Technology) and at least 50 fold for use in RT-PCR.
For accurate OD measurement using a cuvette, dilute RNA in water with a slightly alkaline pH (4). 1 mM NaOH or buffer with a pH > 8 can be used for this purpose. Typically, distilled water has an acidic pH.
Frequent vortexing is hard on hand joints and may lead to injury. Use vortex equipped with a platform with holes for tubes.

The isolated large RNA fraction contains RNA > 150 - 200 bases. This RNA fraction constitutes 80 - 85% of cellular RNA. Expected yields: A) tissues (µg RNA/mg tissue): liver, 5 - 7 µg; kidney, spleen, 3 - 4 µg; skeletal muscle, brain, lung 0.5 - 1.5 µg; placenta, 1 - 3 µg: B) cells (µg RNA/10^6 cells): epithelial cells, 5 - 8 µg; fibroblasts, 3 - 5 µg.
The isolated RNA has a 260/280 ratio of 1.7 to 2.1 and a 260/230 ratio of 1.8 to 2.3. The RIN value of RNA for tissue removed from an animal (or frozen in liquid nitrogen and stored at -70) and immediately processed is > 8.

The isolated small RNA fraction contains RNA < 150 - 200 bases.
The isolated RNA has a 260/280 ratio of 1.6 - 1.9 and a 260/230 ratio of about 1.5.

See also NOTES TO THE PROTOCOL, TROUBLESHOOTING GUIDE AND DNA RECOVERY on Page 2.

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RNAzol® RT
PROTOCOL FOR ISOLATION OF TOTAL RNA.

This protocol yields all classes of RNA in one fraction containing: large nuclear RNA, rRNA, mRNA, small RNA and microRNA down to 10 bases.

Reagents required but not supplied: ethanol, isopropanol, 4-bromoanisole (BAN, optional) and RNase-free water. We recommend the use of disposable polypropylene tubes (see MRC catalog). Tubes from other sources should be tested to ensure integrity during centrifugation at 12,000 g with RNAzol® RT.

The isolation is performed at room temperature and centrifugation at 4 - 28 C.
When the expected yield is < 10µg of RNA, add 1 - 2 µl of Polyacryl Carrier (PC 152) to the initial homogenate/lysate.

1. HOMOGENIZATION.

A. Tissues. Homogenize a tissue sample in a Polytron-type homogenizer or glass-teflon homogenizer using up to 100 mg of tissue per 1 ml of RNAzol® RT. When processing tissues with high DNA content such as spleen, use 50 mg of tissue per 1 ml reagent.
For clarity of presentation, the protocol describes isolation using 1 ml of RNAzol® RT. For the most practical/economical processing of samples, use 1.5 - 2 ml microcentrifuge tubes. For example, use a 0.55 ml aliquot(s) of the homogenate (0.55 ml = 0.5 ml reagent + 50 mg tissue) and freeze the rest of the homogenate for later use. The homogenate can be stored at -20 or -70 C for at least two years.
B. Cells. Cells grown in monolayer should be lysed in a culture dish by addition of RNAzol® RT. Remove culture medium and add at least 1 ml of the reagent per 3.5 cm culture dish (10 cm^2). Pass the lysate through a pipette several times to ensure lysis. Cells grown in suspension should be sedimented first and then lysed by the addition of RNAzol® RT. Add at least 1 ml of RNAzol®RT per 10^7 cells and lyse cells by repeated pipetting.
Washing cells before the addition of RNAzol® RT is not recommended as it may contribute to RNA degradation.
For cells grown in monolayer, use the amount of RNAzol® RT based on the area of the culture dish and not on cell number. An insufficient amount of RNAzol® RT will result in DNA contamination of the isolated RNA.
C. Liquid Samples. Homogenize/lyse liquid samples using 1 ml of RNAzol® RT per 0.4 ml of a liquid sample. For processing a small volume sample, mix the sample with 1 ml of RNAzol® RT and supplement the mixture with water to approach the sample + water volume of 0.4 ml.
D. Samples With High Fat Content. After complete homogenization, centrifuge high-fat samples at 12,000 g for 5 minutes. Excess lipid forms a layer at the top of the tube. Remove most of this layer with a pipette or syringe. Mix the remaining homogenate with 0.4 volumes of water, shake vigorously and continue RNA isolation as described in Step 2.

2. DNA, PROTEIN AND POLYSACCHARIDE PRECIPITATION.

Add to the homogenate/lysate 0.4 ml of water per 1 ml of RNAzol® RT used for homogenization. Shake the resulting mixture vigorously for 15 seconds and store for 5 - 15 minutes. Samples with 100 mg tissue/ml RNAzol® RT require a 15 minute storage at room temperature. Centrifuge sample at 12,000 g for 15 minutes. Following centrifugation, DNA, proteins and most polysaccharides form a semisolid pellet at the bottom of the tube. The RNA remains soluble in the supernatant. Transfer 1 ml of the supernatant (75% of total supernatant volume) to a new tube, leaving a layer of the supernatant above the DNA/protein pellet.
Centrifugation at this and other steps of the protocol can be performed at 4 - 28 C.
The DNA-protein pellet in a sample with 100 mg tissue/ml reagent constitutes about 10% of the total volume of the homogenate-water mixture (for 80 mg/ml it is about 8%). It is safe to collect up to 85% of the supernatant.

3. PHASE SEPARATION (OPTIONAL STEP).

A phase separation step can be incorporated into the basic protocol for total RNA isolation. This additional step is beneficial for samples with a high content of DNA and/or extracellular material. Add 5 µl (0.5% of the supernatant volume) of 4-bromoanisole (MRC, BN 191) to 1ml of the transferred supernatant. Shake the resulting mixture for 15 seconds, store it for 3 - 5 minutes and centrifuge it at 12,000 g for 10 minutes at 4 - 25 C. After centrifugation, residual DNA, protein and polysaccharide precipitate accumulates in the organic phase at the bottom of a tube, while RNA remains soluble in the supernatant.
4-bromoanisole ***cannot*** *be substituted with bromochloropropane or chloroform.*

4. PRECIPITATION OF TOTAL RNA.

Transfer the RNA-containing supernatant obtained in either Step 2 or 3 above to a new tube. Precipitate RNA by mixing 1 ml of the supernatant with 1 ml of isopropanol. Store samples for 10 minutes and centrifuge at 12,000 g for 10 minutes. In most cases, RNA precipitate forms a white pellet at the bottom of a tube. In samples such as spleen, RNA accumulates as a gel-like membrane on the side of a tube. This becomes more visible during washing with ethanol.

5. RNA WASHES. Wash the RNA by mixing the pellet twice with 75% ethanol (v/v). Centrifuge the pellet at 4,000 - 8,000 g for 1 - 3 minutes. When performing isolation in 1.5 ml microcentrifuge tubes, use 0.4 - 0.6 ml of the ethanol solution. For samples processed in larger tubes, use 0.5 ml of alcohol solution per 1 ml of the supernatant used for precipitation. Remove the alcohol solution using a micropipette.

6. RNA SOLUBILIZATION. Dissolve the RNA pellet, without drying, in water or FORMAzol® (MRC, FO 121) to approach an RNA concentration of 1 - 2 µg/ml. For solubilization in water, vortex the RNA pellet at room temperature for 2 - 5 minutes. Tubes and water used for the RNA solubilization should be RNase-free. Solubilization in FORMAzol® requires incubation of RNA for 10 - 20 minutes followed by vortexing or repetitive pipetting.
Drying the RNA pellet is not recommended as this greatly decreases its solubility.
Solubilization of RNA in FORMAzol® can be facilitated by incubation at 55 C for 5 - 15 minutes.
Samples dissolved in FORMAzol® should be diluted at least 5 fold with buffer before application on RNA Chips (Agilent Technology) and at least 50 fold for use in RT-PCR.
For accurate OD measurement using a cuvette, dilute RNA in water with a slightly alkaline pH (4). 1 mM NaOH or buffer with a pH >8 can be used for this purpose. Typically, distilled water has an acidic pH.
Frequent vortexing is hard on hand joints and may lead to injury. Use vortex equipped with a platform with holes for tubes.

The isolated total RNA contains all classes of RNA including rRNA, mRNA, small RNA and micro RNA down to 10 bases long.
Expected yields: A) tissues (µg RNA/mg tissue): liver, 6 - 8µg; kidney, spleen 3 - 4 µg; skeletal muscle, brain, lung 0.5 - 1.5 µg; placenta, 1 - 3 µg: B) cells (µg RNA/10^6 cells): epithelial cells, 5 - 10 µg; fibroblasts, 4 - 6 µg.
The isolated RNA has a 260/280 ratio of 1.7 to 2.1 and a 260/230 ratio of 1.6 to 2.3. The RIN value of RNA for tissue removed from an animal (or frozen in liquid nitrogen and stored at -70) and immediately processed is > 8.

See also NOTES TO THE PROTOCOL, TROUBLESHOOTING GUIDE and DNA RECOVERY on Page 2.

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