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RNAzol BD Reagent

Catalog Number: FP320

Table 1. Components and Storage

Components	Unit Size	Storage	Stability
RNAzol BD Reagent	100 mL	Room temperature	The product is stable for 12 months at room temperature.
BCP	8 mL		

Product Description

RNAzol BD Reagent is a ready-to-use reagent, designed to isolate total RNA, large RNA or small RNA from whole blood, plasma or serum of human or animal origin. The RNA can be used for RNA sequencing and gene expression analysis, as well as RT-PCR, qRT-PCR, microarrays, poly A+ selection or other molecular biology applications. In addition, the reagent allows for simultaneous isolation of RNA and DNA from the same biological sample. The outstanding effectiveness and versatility of RNAzol BD Reagent makes it the most advanced and reliable reagent in this category.

RNAzol BD Reagent provides an unsurpassed yield of either total RNA, large RNA (> 150 - 200 bases) or small RNA (< 150 - 200 bases). The small RNA constitutes about 15 - 30% of the total RNA. RNAzol BD provides an opportunity to isolate and investigate all RNA present in whole blood, and not a small fraction as isolated by other methods.

RNAzol BD Reagent is a monophase solution containing acidic phenol and guanidine thiocyanate. A blood, plasma or serum sample is lysed in RNAzol BD and RNA is separated from DNA, proteins, polysaccharides and other cellular components by phase separation. Pure RNA is precipitated from the aqueous supernatant by the addition of isopropanol. The RNA pellet is washed and solubilized. Use 1 ml of RNAzol BD Reagent to process 0.5 ml of blood in less than one hour.

Required materials not supplied

- Isopropanol
- 75% Ethanol
- RNase-free water
- Acetic acid
- Centrifuge capable of reaching 12,000 × g

PROTOCOLS FOR RNA ISOLATION

Isolation of total RNA - Abbreviated Protocol.

1. Lysis	- 1 ml blood + 2 ml RNAzol BD + 30 µl acetic acid, shake 30 sec, 15 min at 37-40°C.
2. DNA/protein removal	- 3 ml lysate + 150 µl BCP, mix, store 15 min at RT, 12,000 g x 15 min at RT.
3. Total RNA precipitation	- 1.5 ml supernatant + 1.5 ml isopropanol, mix, store 15 min at RT, 12,000 g x 15 min.
4. RNA washes	- 0.8 ml 75% ethanol, mix, 12,000 g x 1-3 min at RT; wash twice.
5. RNA solubilization	- RNase-free water.

Except for lysate incubation in Step 1, centrifugation and all other steps are performed at room temperature.

Isolation of large RNA and small RNA in separate fractions - Abbreviated Protocol.

1. Lysis	- 1 ml blood + 2 ml RNAzol BD + 30 µl acetic acid, shake 30 sec, 15 min at 37-40°C.
2. DNA/protein removal	- 3 ml lysate + 150 µl BCP, mix, store 15 min at RT, 12,000 g x 15 min at RT.
3. Large RNA precipitation	- 1.5 ml supernatant + 0.525 ml isopropanol, mix, store 15 min at RT, 12,000 g x 15 min.
4. Large RNA washes	- 0.8 ml 75% ethanol, mix, 12,000 g x 1-3 min at RT; wash twice.
5. Large RNA solubilization	- RNase-free water.
6. Small RNA precipitation	- large RNA supernatant + 0.975 ml isopropanol, mix, store 30 min, 12,000 g x 15 min.
7. Small RNA washes	- 0.8 ml 70% isopropanol, mix, 12,000 g x 3 min at RT; wash twice.
8. Small RNA solubilization	- RNase-free water.

Except for lysate incubation (Step 1) and small RNA precipitation (Step 6), all steps are performed at room temperature.

1. LYSIS

Collect whole blood into a vacuum tube (preferentially with EDTA as an anticoagulant) and immediately transfer blood aliquots to tubes containing RNAzol BD and acetic acid. Add 1 ml of blood to 2 ml of the reagent and 30 µl of glacial acetic acid. Tightly cover the tube and vigorously shake the blood - RNAzol BD mixture for 30 seconds.

Note: Vigorous shaking is important to ensure efficient removal of DNA from the aqueous phase. The resulting lysate can be stored at -70 °C for at least 2 years and at -20 °C at least 6 months. After removing stored lysate from the freezer, thaw samples with shaking.

Alternatively, store aliquots of blood at -70 °C. For example, transfer 1 ml aliquots of blood into 5 ml tubes and store at -70 °C. For processing, add 2 ml of RNAzol BD and 30 µl of glacial acetic acid to the frozen blood without thawing. Place the tubes in warm water (> 55 °C) and thaw the contents with shaking.

Note: Do not thaw blood samples without the reagent. This will lead to RNA degradation.

2. DNA, PROTEIN AND POLYSACCHARIDE REMOVAL

This protocol describes processing 3 ml of the lysate (1 ml blood + 2 ml RNAzol BD) using 5 ml tubes. Incubate the lysate for 15 min at 37-40 °C (or room temperature for a frozen lysate after thawing). Following incubation, supplement 3 ml of lysate with 150 µl of BCP (1-bromo-3-chloropropane). Tightly cover the tube and vigorously shake the mixture for 30 seconds. Store the sample for 15 min at room temperature and centrifuge the lysate at 12,000 g for 15 min at room temperature. Following centrifugation, DNA, proteins and polysaccharides are sequestered in the interphase and phenol phase at the bottom of the tube. RNA remains soluble in the aqueous supernatant at the top of the tube.

Note: Centrifugation of the lysate at temperatures below 15 °C results in a decrease of RNA yield.

Remove the remaining aqueous phase and store the interphase/phenol phase at 4 or -20 °C for DNA isolation.

3. RNA PRECIPITATION

Step 3A isolates total RNA in one precipitation step. Steps 3B and 3C isolate large RNA and small RNA in separate fractions in two sequential precipitation steps.

3A. TOTAL RNA PRECIPITATION

This step precipitates total RNA containing large nuclear RNA, rRNA, mRNA, tRNA, small RNA and microRNA.

Transfer 1.5 ml of the aqueous supernatant from Step 2 to a new tube. Leave a 2 - 3 mm layer of supernatant above the interphase/phenol phase to avoid disturbing the lower phases. Mix the transferred supernatant with an equal volume of isopropanol (1.5 ml) to precipitate total RNA. Store the sample for 15 min at room temperature and centrifuge at 12,000 g for 15 min at room temperature. RNA precipitate forms a white gel-like pellet at the bottom of a tube. Immediately after centrifugation, the RNA pellet adheres to the side of the tube and most of the supernatant can be removed by decanting. Remove the remaining supernatant from the tube with a micropipette.

Note: It is safe to collect up to 85% of the aqueous supernatant without disturbing the interphase/phenol phase.

3B. LARGE RNA PRECIPITATION

This step precipitates RNA > 150 - 200 bases, including large nuclear RNA, rRNA and mRNA. Due to multiple splicing sites, some small RNA can be detected in both the large and small RNA fractions.

Transfer 1.5 ml of the aqueous supernatant from Step 2 to a new tube. Leave a 2 - 3 mm layer of the supernatant above the interphase/phenol phase to avoid disturbing the lower phases. Mix the transferred supernatant with 0.525 ml (0.35 volumes) of isopropanol to precipitate the large RNA fraction. Store sample for 15 min at room temperature and centrifuge at 12,000 g for 15 min at room temperature. RNA precipitate forms a clear gel-like pellet at the bottom of a tube, which becomes white and visible during the ethanol wash step. Immediately after centrifugation, the RNA pellet adheres to side of the tube and most of the supernatant can be removed by decanting. Remove the remaining supernatant from the tube with a micropipette. Retain the removed supernatant in a new tube and store at -20 or -70 °C for subsequent isolation of the small RNA fraction from this isopropanol-supernatant.

3C. SMALL RNA PRECIPITATION.

This step precipitates RNA < 150 - 200 bases including small rRNA, tRNA, small RNA and microRNA down to 10 bases.

Supplement the supernatant obtained in Step 3B with 0.975 ml of isopropanol. Mix well and store samples at room temperature or 4 °C for 30 min. Sediment precipitated RNA at 12,000g for 15 min at room temperature. RNA forms a white pellet at the bottom of a tube.

4. RNA WASH

When using larger volume tubes, it is beneficial to transfer the RNA pellet into a microcentrifuge tube for the wash steps. Add 0.8 ml of wash solution. Gently resuspend the RNA pellet and transfer it to a microcentrifuge tube using a 1 ml pipette tip. Before transfer, cut the end of the tip to create a sufficiently large opening to collect the RNA pellet into the tip.

4A. WASHING TOTAL RNA OR LARGE RNA FRACTION PELLET.

Add 0.8 ml of 75% ethanol (v/v) to the RNA pellet. Mix the pellet in the wash solution and centrifuge at 12,000 g for 1 - 3 min. After each wash, use a pipette tip to remove the remaining wash solution. Perform two washes.

4B. WASHING SMALL RNA FRACTION PELLET.

Add 0.8 ml of 70% isopropanol (v/v) to the RNA pellet. Mix the pellet and store for 2 - 3 min, followed by centrifugation at 12,000 g for 2 - 3 min. After each wash, use a pipette to remove the remaining wash solution. Perform two washes.

5. RNA SOLUBILIZATION.

Dissolve the RNA pellet, without drying, in RNase-free water. Vortex the RNA pellet at room temperature for 3 - 5 min.

Note: Drying the RNA pellet is not recommended as this greatly decreases its solubility.

Total RNA precipitation yields all classes of RNA. Expected yield is 8 - 22 µg of total RNA and 5 - 16 µg of the large RNA fraction per 1 ml of human blood with a normal white cell count. Human blood with an elevated white cell count may yield >20 µg total RNA/ml blood. RNAzol BD isolates 20 - 90 µg of RNA/ml from animal blood.

The total RNA or large RNA fraction has a 260/280 ratio of 1.7 - 2.0 and a 260/230 ratio of about 2. The RIN value of human blood mixed with the reagent immediately after collection is about 7.0 - 7.5 for total RNA and 7.0 - 8.5 for the large RNA fraction. The small RNA fraction has a 260/280 ratio of about 1.7 and 260/230 ratio of about 1.5. Typically for human blood, the small RNA fraction constitutes 15 - 30% of the total RNA. This corresponds to 2 - 5 µg of small RNA per 1 ml blood.

NOTES FOR RNA ISOLATION

1. The mixing of blood with RNAzol BD immediately after collection is critical to the integrity and yield of RNA.
2. RNA isolation can be interrupted and samples can be stored as indicated below:

   A sample lysed in RNAzol BD (Step 1) can be stored at room temperature for at least 3 hours, at 4 °C for at least 24 hours, at -20 °C for at least 6 months, and at -70 °C for at least 2 years. To thaw samples, incubate them at 37 - 50 °C for 5 – 10 min with intermittent mixing.

   The RNA precipitate can be stored in 75% ethanol overnight at room temperature, for at least one week at 4 °C, or at least one year at -20 °C.
3. When isolating low quantities of RNA (< 5 µg), residual supernatant adhering to the side of the tube can lower the A260/280 ratio. After centrifugation to obtain the RNA pellet, remove the supernatant from the tube. Briefly centrifuge the tube again to collect residual supernatant and remove it with a micropipette prior to washing steps. Similarly, centrifuge and remove any residual alcohol after the final wash step.
4. For RNA isolation from plasma or serum: Add 1 ml of plasma or serum to 2 ml of RNAzol BD and 10 µl of glacial acetic acid. Vigorously shake for 15 - 30 seconds. Add 150 µl of BCP to the lysate, shake vigorously for 15 - 30 seconds and store for 15 min at room temperature. Centrifuge at 12,000 g for 15 min. Proceed with total RNA precipitation (Step 3A) and wash (Step 4A). Solubilize the RNA in 10 - 20 µl of water. RNA yield is below the Nanodrop spectrophotometry sensitivity. Use approximately 50% of the RNA product in RT-PCR to obtain detectable reaction product in 25 - 40 cycles, depending on primers and target abundance.

RNA ISOLATION TROUBLESHOOTING GUIDE

Low yield. a) incomplete solubilization of the final RNA pellet, b) after collection, blood sample was not immediately mixed with the reagent, c) too much blood was used for the volume of the reagent, d) RNAzol BD - blood - BCP lysate was centrifuged at a temperature < 15 °C.

260/280 ratio < 1.6. a) ineffective wash of the RNA pellet. Mix samples more vigorously and store samples with 75% ethanol for 2 - 3 min at room temperature, b) acidic water was used for the OD measurement, c) incomplete solubilization of the RNA pellet, d) insufficient volume of reagent was used for lysis.

RNA degradation. a) blood was not immediately mixed with the reagent, b) blood was not immediately frozen after collection, c) aqueous solutions or tubes used for solubilization of RNA were not RNase-free.

DNA contamination. a) too much blood was used for the volume of the reagent, b) RNAzol BD - blood - BCP lysate was centrifuged at a temperature < 15 °C.

PROTOCOL FOR DNA ISOLATION

The protocol for DNA isolation with RNAzol BD is based on re-extraction of DNA sequestered into the interphase/phenol phase during RNA isolation. This method allows for analysis of RNA and DNA derived from the same sample. The isolated DNA can be used for PCR, sequencing and other molecular biology applications.

Isolation of DNA - Abbreviated Protocol.

DNA isolation from the phenol phase/interphase derived from 1 ml of whole blood lysed in 2 ml of RNAzol BD.

1. DNA re-extraction	- phenol phase/interphase from1ml blood + 1 ml 4M guanidine thiocyanate + 100 µl 3M NaOH shake, store 10 min at RT.
2. DNA separation	- add 150 µl BCP, shake, 12,000 g x 10 min at RT.
3. DNA precipitation	- aqueous supernatant + 1 vol isopropanol, mix, store 15 min at RT, 12,000 g x 15 min at RT.
4. DNA washes	- 75% ethanol, mix, 12,000 g x 1 - 3 min at RT.
5. DNA solubilization	- water or TE buffer.

This procedure is carried out at room temperature. Calculation of solution volumes is based on the volume of blood used for RNA isolation.

1. DNA ISOLATION

Remove the remaining aqueous phase overlying the interphase/phenol phase obtained from Step 2 of the RNA isolation procedure. Extract DNA by adding to the interphase/phenol phase derived from 1 ml of blood: 1 ml of 4M guanidine thiocyanate, 0.1 ml of 3M NaOH. Tightly cover the tube and vigorously shake for 30 seconds to obtain a fine suspension. Store the mixture for 10 minutes at room temperature and shake for 30 seconds at the end of incubation.

Note: This step solubilizes the DNA precipitated during RNA isolation.

2. DNA SEPARATION

Add to the extraction mixture 150 µl of BCP, tightly cover the tube and vigorously shake for 30 seconds. Centrifuge at 12,000 g for 10 min at room temperature. During this step, DNA is sequestered into the upper aqueous supernatant while proteins, carbohydrates and other molecules remain in the lower phenol phase.

3. DNA PRECIPITATION

Transfer 1 ml the aqueous supernatant into a new 5ml tube and precipitate DNA by mixing with 1 volume of isopropanol. Store at room temperature for 15 min and sediment the DNA precipitate at 12,000 g for 15 min at room temperature.

4. DNA WASH

It is beneficial to transfer the DNA pellet from a large tube into a microcentrifuge tube. After removing the supernatant, gently suspend the DNA pellet in 0.6 - 0.8 ml of 75% ethanol in the large tube. Transfer the DNA pellet to a microcentrifuge tube using a 1 ml pipette tip. Before transfer, cut the end of the tip to create a sufficiently large opening to collect the pellet into the tip.

After transfer, sediment the DNA pellet by centrifugation at 12,000 g for 1 - 3 min at 4 - 25 °C. Wash the DNA pellet a second time with 75% ethanol and centrifuge at 12,000 g for 1 - 3 min.

5. DNA SOLUBILIZATION

Dissolve the DNA pellet in water or a buffer with pH > 7.5 by vortexing or passing the pellet through a micropipette. Add an adequate amount of solution to approach a DNA concentration of 0.1 - 0.3 µg DNA/µl.

6. QUANTITATION OF DNA.

Calculate the DNA content assuming that one A260 unit equals 50 µg double-stranded DNA/ml.

A preparation of DNA isolated from whole blood contains predominantly DNA >100 kb. The isolated DNA is free of RNA and proteins and has a 260/280 ratio >1.8 and 260/230 ratio 1.7 - 2. For human blood, typical recovery of DNA is 35 - 45 µg DNA/ml.