PRODUCT: **RNAzol® BD COLUMN KIT** January 2018
Catalog No: RC 292
Storage: Store at room temperature.

PRODUCT DESCRIPTION.

The RNAzol® BD Column Kit isolates 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 Kit allows for simultaneous isolation of RNA and DNA from the same biological sample. The outstanding effectiveness and versatility of the RNAzol® BD Column Kit makes it the most advanced and reliable kit in this category.

RNAzol® BD has been used to determine RNA content in human blood, which averages 14 µg/ml of whole blood and ranges from 6 - 22 µg/ml (5). These levels of RNA are significantly higher than previously reported with other methods that average only 2 - 5 µg of total RNA per ml of human blood. RNAzol® BD 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 in blood. 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 isolates 20 - 150 µg of RNA/ml of animal blood depending on the species.

RNAzol® BD (US patents, 1, 2) is based on an improved single-step method to provide the highest yield and purity of isolated RNA, and is more effective than previous reagents (3, 4). RNAzol® BD 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 other molecules in a single-step by precipitating DNA, proteins, polysaccharides and other cellular components. RNA remains in an aqueous supernatant that is mixed with alcohol and applied on a column to further purify the RNA. Following alcohol washes, pure RNA is eluted from the column with water. Use 1 ml of RNAzol® BD to process 0.5 ml of blood in less than one hour.

KIT SPECIFICATIONS	**QUANTITY**
RNAzol® BD	50 ml
Columns with Collection tubes	50
Wash tubes	50
Elution tubes	50
RNase-free water	10 ml

Supplies allow for 50 isolations of total RNA, or 50 isolations large RNA and small RNA in separate fractions.
Column capacity is 800 µl of solution and at least 300 µg of RNA.

Reagents required but not supplied: 1-bromo-3-chloropropane (BCP, MRC cat. no. BP 151), acetic acid, isopropanol and ethanol. We recommend the use of disposable polypropylene tubes (see MRC catalog). Other tubes should be tested to ensure integrity during centrifugation at 12,000 g with RNAzol® BD.

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

SPECIAL HANDLING PRECAUTIONS.
RNAzol® BD contains phenol (poison, corrosive) and guanidine thiocyanate (irritant). CAUSES BURNS. Can be fatal. When working with RNAzol® BD, use gloves and eye protection (face shield, safety goggles). Do not get on skin or clothing. Avoid breathing fumes. Read the warning 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.

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 minutes 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.
- RNA solubilized in water should be stored at -70 C.

3. When isolating low quantities of large RNA in a pellet (< 5 µg), residual supernatant adhering to the side of the tube can lower the A260/280 ratio. After removing the supernatant, briefly centrifuge the tube 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 minutes at room temperature. Centrifuge at 12,000 g for 15 minutes. Obtain the aqueous supernatant and proceed with column purification of total RNA. RNA yield may be below the Nanodrop spectrophotometry sensitivity. Use approximately 50% of the RNA product in RT-PCR to obtain detectable reaction product in 25 - 50 cycles, depending on primers and target abundance.

RNA ISOLATION TROUBLESHOOTING GUIDE.

Low yield. a) incomplete solubilization of the final RNA pellet or insufficient elution of RNA from the spin column; b) after collection, blood sample was not immediately mixed with the reagent; c) insufficient volume of reagent was used for lysis; 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 minutes 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.

REFERENCES.

1. Chomczynski P. Reagents and methods for isolation of purified RNA. US Patent 7,794,932.
2. Chomczynski P. Reagents for isolation of purified RNA. US Patent 8,367,817. International Patents Pending.
3. Chomczynski P, and Sacchi N. (1987) Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162, 156-159.
4. 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.
5. Chomczynski, P., et. al. (2016) Inter-individual differences in RNA levels in human peripheral blood. PLoS ONE 11(2): e0148260. doi:10.1371/journal.pone.0148260.
6. 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.

The RNAzol® BD Column Kit method can be cited by referring to: RNAzol® BD Column Kit Brochure, 2018, Molecular Research Center, Inc. Cincinnati, OH. RNAzol® and FORMAzol® are registered trademarks of Molecular Research Center, Inc.

RNAzol BD® COLUMN KIT
PROTOCOL FOR ISOLATION OF TOTAL RNA

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

Protocol Summary.

1. Lysis	- 1 ml blood + 2 ml RNAzol® BD + 27 µl acetic acid, shake 30 sec, 15 min at 37 - 40 C.
2. DNA/protein removal	- mix 3 ml lysate + 150 µl BCP, store 15 min at RT, 12,000 g x 15 min at RT.
3. Total RNA purification	- 1 vol supernatant + 1 vol isopropanol, apply on column, store 2 - 5 min, 12,000 g x 20 sec.
4. RNA washes	- 2X: 0.3 ml 95% ethanol, 12,000 g x 20 sec (or 1 - 2 min for RNA yield < 10µg).
5. RNA solubilization	- 2X: 20 - 40 µl water, store 1 min, 12,000 g x 20 sec.

Except for lysate incubation in Step 1, centrifugation and all other 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 27 µl of glacial acetic acid. Tightly cover the tube and vigorously shake the blood - RNAzol® BD mixture for 30 seconds. Please see Note 4 for RNA isolation from plasma or serum.

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 for at least 6 months. After removing stored lysate from the freezer, thaw samples with shaking.

Alternatively, store aliquots of fresh 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 27 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.

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

Blood adheres to glass and plastic surfaces during pipetting. This results in 5 - 10% loss in blood volume. A correction can be made by determining the weight of aliquoted blood samples. Human blood density is 1.06 g/ml.

2. DNA, PROTEIN AND POLYSACCHARIDE PRECIPITATION.

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 minutes 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 minutes at room temperature and centrifuge the lysate at 12,000 g for 15 minutes 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.

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. COLUMN PURIFICATION OF TOTAL RNA.

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 phase. Add 1 volume of isopropanol and mix it in the tube.

Supernatant - isopropanol volumes ≤ 0.8 ml: Transfer the supernatant - isopropanol mixture to the column, cap the column and store for 2 - 5 min at room temperature. Centrifuge at 12,000 g for 20 seconds. The total RNA is retained on the column.

Supernatant - isopropanol volumes > 0.8 ml: Transfer 0.6 - 0.8 ml of the supernatant - isopropanol mixture to the column, cap the column and store for 2 - 5 minutes at room temperature. Centrifuge at 12,000 g for 20 seconds. Remove the column from the collection tube and discard the pass-through solution. Re-insert the column in the collection tube. Apply the next aliquot of the supernatant - isopropanol mixture on the column and centrifuge at 12,000 g for 20 seconds. It is not necessary to wait an additional 2 - 5 min between each application of more solution on the column. Repeat the procedure until the entire sample is processed. The total RNA is retained on the column.

The loading limit on the column is at least 300 µg RNA.

Excess supernatant - isopropanol mixture can be stored at -20 or -70 C for at least one year.

4. TOTAL RNA WASH.
Insert the column into a clean, labeled wash tube. Apply 0.3 ml of 95% ethanol to the column and centrifuge at 12,000 g for 20 seconds. Repeat the ethanol wash step a second time.
When isolating a small amount of RNA (< 10 µg) the second spin should last 1 minute to evaporate any residual ethanol from the column.

5. TOTAL RNA ELUTION.
Insert the column into a clean, labeled elution tube. Apply 20 - 40 µl of RNase-free water near the bottom of the column. Store for 1 minute to hydrate the RNA and elute RNA from the column by centrifugation at 12,000 g for 20 seconds. Repeat the elution using a fresh volume of 20 - 40 µl of RNase-free water (total elution volume = 40 - 80 µl). Remove the column from the elution tube, cap the tube and vortex the eluted RNA solution. Measure the RNA content and store the RNA solution at -70 C.
Decreasing the total elution volume may increase the solubilized RNA concentration and/or diminish the RNA yield. Another option to increase the eluted RNA concentration is to reapply the eluted RNA to the column for a second elution pass. This option may work well for yields less than 10 µg RNA.

The purified total RNA contains all classes of RNA including high molecular weight untranslated RNA, rRNA, mRNA, small RNA and microRNA down to 10 bases long. Expected yield is 8 - 22 µg of total RNA 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 - 150 µg of RNA/ml from animal blood depending on the species.

The results of RT-qPCR can be calculated per µg of RNA and per ml of blood. Due to the wide range of RNA content in whole blood, calculation of gene activity per ml of blood more accurately reflects accumulation of a gene product in the circulation (5). This calculation is particularly important for evaluating expression of signaling molecules or cytokines such as interleukins, TGFs and TNFs.

The total RNA has a 260/280 ratio of 1.7 - 2.0 and a 260/230 ratio of about 2. The RIN value of RNA from human blood mixed with the reagent immediately after collection is about 7.0 - 8.5 for total RNA.
For accurate OD measurement using a cuvette, use diluent with a slightly alkaline pH (6). Use 3 mM NaOH or a buffer with a pH >8. Typically, distilled water has an acidic pH.

RNAzol BD® COLUMN KIT
PROTOCOL FOR ISOLATION OF LARGE RNA AND SMALL RNA

This protocol yields two fractions of RNA: (1) large RNA > 150 - 200 bases including long non-coding RNA, rRNA mRNA, and (2) small RNA < 150 - 200 bases including tRNA, small rRNA, and microRNA down to 10 bases. Due to multiple splicing sites, some small RNA can be detected in both the large and small RNA fractions.

Protocol Summary.

1. Lysis	- 1 ml blood + 2 ml RNAzol® BD + 27 µl acetic acid., shake 30 sec, 15 min at 37 - 40 C.
2. DNA/protein removal	- 3 ml lysate + 150 µml BCP, mix, store 15 min at RT, 12,000 g x 15 min at RT.
3. Large RNA precipitation	- mix 1 vol supernatant + 0.35 vol isopropanol, store 15 min at RT, 12,000 g x 15 min.
4. Large RNA washes	- 0.4 - 0.8 ml 75% ethanol, mix, 12,000 g x 1 - 3 min at RT; wash twice.
5. Large RNA solubilization	- water or FORMAzol®.
6. Small RNA purification	- Step 3 supernatant + 0.481 vol isopropanol, apply on column, 15 min, 12,000 g x 20 sec.
7. Small RNA washes	- 2X: 0.3 ml 95% ethanol, 12,000 g x 20 sec (or 1 - 2 min for RNA yield < 10µg).
8. Small RNA solubilization	- 2X: 20 - 40 µl water, store 1 min, 12,000 g x 20 sec.

Except for lysate incubation in Step 1, centrifugation and all other 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 27 µl of glacial acetic acid. Tightly cover the tube and vigorously shake the blood - RNAzol® BD mixture for 30 seconds. Please see Note 4 For RNA Isolation from plasma or serum.

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 for at least 6 months. After removing stored lysate from the freezer, thaw samples with shaking.

Alternatively, store aliquots of fresh 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 27 µl of acetic acid to the frozen blood without thawing. Place the tubes in warm water (> 55 C) and thaw the contents with shaking.

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

Blood adheres to glass and plastic surfaces during pipetting. This results in 5 - 10% loss in blood volume. A correction can be made by determining the weight of aliquoted blood samples. Human blood density is 1.06 g/ml.

2. DNA, PROTEIN AND POLYSACCHARIDE PRECIPITATION.

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 minutes at 37 - 40 C (or room temperature for frozen samples 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 minutes at room temperature and centrifuge the lysate at 12,000 g for 15 minutes 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.

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. PRECIPITATION OF THE LARGE RNA FRACTION.

This step precipitates large RNA > 150 - 200 bases, including large nuclear RNA, rRNA and mRNA.

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 phase. Mix the transferred supernatant with 0.35 volumes of isopropanol (0.525 ml) to precipitate the large RNA fraction. Store the sample for 15 minutes at room temperature and centrifuge at 12,000 g for 15 minutes 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 supernatant in a new tube and store at -20 or -70 C for subsequent isolation of the small RNA fraction from this isopropanol-supernatant.

Samples containing < 0.5 ml of blood require addition of 1 - 2 µl of Polyacryl Carrier (PC 152) to improve RNA recovery and sample handling.
1 ml of the supernatant is equivalent to 0.55 ml of blood.
1 ml of the supernatant-isopropanol solution is equivalent to 0.407 ml of blood in the large RNA fraction precipitation.

4. WASHING THE LARGE RNA FRACTION PELLET.
Use 75% ethanol (v/v) for wash steps. Use 0.4 ml for washes in microcentrifuge tubes; for washes in larger tubes use 0.2 - 0.5 ml of ethanol wash per 1 ml of supernatant used for precipitation. Mix the pellet in the wash solution and centrifuge at 12,000 g for 1 - 3 minutes. After each wash, use a pipette tip to remove the remaining wash solution. Perform two washes.

5. SOLUBILIZING THE LARGE RNA PELLET
Dissolve the large RNA pellet, without drying, in water or FORMAzol® (MRC, FO 121). For solubilization in water, vortex the RNA pellet at room temperature for 3 - 5 minutes. Tubes and water used for the RNA solubilization should be RNase-free. For solubilization in FORMAzol®, mix the solution for 10 minutes on a vortex platform. Avoid injury to hand joints by using a vortex equipped with a platform attachment to hold the tubes. Drying the RNA pellet is not recommended as this greatly decreases its solubility.

Expected yield is 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 results of RT-qPCR can be calculated per µg of RNA or per ml of blood. Due to the wide range of RNA content in whole blood, calculation of gene activity per ml of blood more accurately reflects accumulation of a gene product in the circulation (5). This calculation is particularly important for evaluating expression of signaling molecules or cytokines such as interleukins, TGFs and TNFs.

The 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 large RNA from human blood mixed with the reagent immediately after collection is about 7.0 - 8.5 for the large RNA fraction.
For accurate OD measurement using a cuvette, use diluent with a slightly alkaline pH (6). Use 3 mM NaOH or a buffer with a pH >8. Typically, distilled water has an acidic pH.

6. COLUMN PURIFICATION OF THE SMALL RNA FRACTION.
Supplement the supernatant collected in Step 3 with 0.481 volumes of isopropanol per 1 volume of the solution and mix in the tube. Apply up to 0.8 ml of the mixture to a clean column and store for 15 minutes at room temperature. Centrifuge at 12,000 g for 20 seconds. Remove the column from the collection tube and discard the pass-through solution. Re-insert the column back into the collection tube. Apply the next aliquot of the mixture onto the column and repeat the procedure until the entire sample is processed. It is not necessary to wait an additional 15 minutes between each application of more solution on the column.

7. SMALL RNA WASH.
Insert the column into a clean, labeled, wash tube. Apply 0.3 - 0.4 ml of 100% ethanol on the column and centrifuge at 12,000 g for 20 seconds. Repeat the ethanol wash step a second time.
When isolating a small amount of RNA (< 10 µg), extend the second spin for 1 minute to allow for evaporation of any residual alcohol from the column.

8. SMALL RNA ELUTION.
Insert the column into a clean, labeled elution tube. Apply 20 - 40 µl of RNase-free water near the bottom of the column. Store for 1 minute to hydrate the RNA and elute the RNA from the column by centrifuge at 12,000 g for 20 seconds. Repeat the elution using a fresh volume of 20 - 40 µl of RNase-free water (total elution volume = 40 - 80 µl). Remove the column after the second elution, cap the tube and vortex the eluted RNA solution. Measure the RNA content and store the RNA solution at -70 C.
Decreasing the total elution volume may increase the solubilized RNA concentration and/or diminish the RNA yield. Another option to increase the eluted RNA concentration is to reapply the eluted RNA to the column for a second elution pass. This option may work well for yields less than 10 µg RNA.

The purified small RNA fraction contains small rRNA, tRNA and microRNA down to 10 bases. Expected yield is about 15 - 30 % of the total RNA yield, corresponding to about 2 - 5 µg of small RNA per 1 ml of human blood. The small RNA fraction has a 260/280 ratio of about 1.7 and a 260/230 ratio of about 1.5.

ISOLATION OF DNA BY RNAzol® BD.

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. The isolated high-molecular weight DNA is not a good substrate for restriction enzymes.

Reagents required, but not supplied: 1-bromo-3-chloropropane (BCP, MRC cat. no. BP 151), isopropanol, ethanol, 4 M guanidine thiocyanate solution (MRC cat. no. GT 192), Polyacryl Carrier (MRC cat. no. PC 152) and 3M NaOH.

Protocol Summary.

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	- interphase/phenol phase from 1 ml blood + 1 ml 4M guanidine thiocyanate + 100 µl 3M NaOH - add 5 µl carrier, shake, store 10 min at RT, shake.
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.

Calculation of solution volumes is based on the volume of blood used for RNA isolation.

1. DNA ISOLATION.

Remove the remaining aqueous phase on top of 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 DNA Extraction Solution (MRC cat. no. GT 192, 4M guanidine solution), 0.1 ml of 3M NaOH and 2 µl of Polyacryl Carrier (MRC cat. no. PC 152). 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.

This step solubilizes the DNA precipitated during RNA isolation.

Use a smaller scale processing to quantify DNA. Transfer a 1 ml aliquot of this extraction mixture into a 1.5 ml microcentrifuge tube and process the mixture as described below in Note 3 for DNA 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 minutes at room temperature. During this step, DNA is sequestered into the upper aqueous supernatant while proteins, carbohydrates and other molecules remain in the 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 minutes and sediment the DNA precipitate at 12,000 g for 15 minutes at room temperature.

1 ml of the supernatant is equivalent to 0.75 ml of blood.

A volume of supernatant used for precipitation also can be estimated by its weight, 1.10 g/ml.

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 minutes at 4 - 25 C. Wash the DNA pellet a second time with 75% ethanol and centrifuge at 12,000 g for 1 - 3 minutes.

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. For small scale isolations use 150 µl water to dissolve the DNA pellet. Depending on the intensity of vortexing, it takes 15 - 30 minutes to dissolve the DNA pellet derived from up to 2 ml of blood. Larger DNA pellets may require a longer dissolution time.

For faster dissolution, add NaOH to a final concentration of 0.5mM. In 0.5 mM NaOH, OD of DNA at 260 remains the same as in water while the 260/280 ratio increases by 10% and the 260/230 ratio decreases by 10%.

6. QUANTITATION OF DNA.

Calculate the DNA content assuming that one A260 unit equals 50 µg of double-stranded DNA/ml. For calculation of the cell number in samples, assume that the amount of DNA per 10^6 diploid cells of human, rat and mouse origin equals 6.6 µg, 6.5 µg and 5.8 µg, respectively.

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. 95 - 100 % recovery of DNA is achieved by precipitating DNA in a 1.5 ml microcentrifuge tube (Note 3).

NOTES FOR DNA ISOLATION

1. If necessary, the interphase/phenol phase can be stored at 4 C overnight. Longer storage of the interphase/phenol phase significantly decreases DNA yield. Do not store samples at room temperature. Alternatively, store the phenol phase/interphase at -20 or -70 C. To thaw the samples, add 4M guanidine thiocyanate and 3M NaOH. Hand shake the sample in 30 - 40 C water until thawed and immediately perform DNA isolation. Storage of the phenol phase at room temperature decreases DNA yield.
2. DNA samples suspended in 75% ethanol can be stored at 4 C for a longer period of time (months). Samples solubilized in water can be stored overnight at 4 C. For prolonged storage, store samples in 1mM Na-EDTA.
3. For a small scale isolation, transfer a 1 ml aliquot of the phenol - guanidine - NaOH extraction mixture into a 1.5 ml microcentrifuge tube. 1 ml of this mixture contains 0.41 ml blood. Supplement the mixture with 61.5 µl BCP, shake for 30 seconds and centrifuge at 12,000 g for 10 minutes at room temperature. Transfer the resulting aqueous supernatant to a new tube, add an equal volume of isopropanol and mix. Store samples for 15 minutes at room temperature and sediment the precipitated DNA at 12,000g for 15 minutes at room temperature. Wash the DNA pellet with 75% ethanol and centrifuge at 12,000 g for 1 - 3 minutes at room temperature. Dissolve the DNA pellet in 150 µl water by vortexing for 15 minutes. Use a vortex platform to hold the tubes. Using this protocol, 95 - 100 % of DNA is recovered from blood samples used for RNA extraction.