PROTOCOL

ambion® | RNA
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RiboPure™-Blood Kit

life technologies™

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RiboPure™-Blood Kit

(Part Number AM1928)

Protocol

I. Introduction

IMPORTANT

Before using this product, read and understand the "Safety Information" in the appendix in this document.

A. Background

The RiboPure™-Blood Kit contains reagents and materials for isolation of high quality total RNA from anticoagulated whole blood. Blood sample volumes from 300 µL to 500 µL per prep can be processed, and the kit contains reagents and materials for 40 preps. Blood samples can be processed immediately, or they can be stored in RNA*later*® Solution (provided with the kit) for a few days at ambient temperature or for prolonged periods at –20°C prior to RNA extraction. The RiboPure-Blood Kit RNA isolation procedure consists of two parts:

1. Cell lysis in a guanidinium-based solution and initial purification of the RNA by phenol/chloroform extraction
2. Final RNA purification by solid-phase extraction on a glass fiber filter

The kit includes DNA-*free*™ reagents for the enzymatic removal of genomic DNA. After the DNase digestion step, the DNase is removed with a simple procedure that inactivates and removes DNase without organic extraction, heat inactivation, or alcohol precipitation.

RNA can be isolated from whole blood in ~15 min using the RiboPure-Blood Kit. Adding the DNA removal treatment increases the procedure time by ~35 min (mostly incubation time). Yields of total RNA are typically ~2–4 µg from 0.5 mL whole blood from normal healthy donors.

This kit can also be used for isolating total RNA from the white blood cell fraction of blood or "buffy coat".

B. Reagents and Materials Included in the Kit and Storage

This kit contains reagents for 40 RNA isolations from 300–500 µL whole blood.

Kit components and storage conditions

Amount	Component	Storage
25 mL	Elution Solution	4°C
40 mL	Lysis Solution	4°C
25 mL	Acid-Phenol:Chloroform	4°C
5 mL	Sodium Acetate Solution	4°C
35 mL	Wash Solution 1	room temp/4°C*
70 mL	Wash 2/3 Concentrate Add 56 mL 100% ethanol before use	room temp/4°C*
100 mL	RNA*later*® Solution	room temp
40	Filter Cartridges	room temp
80	Collection Tubes	room temp
100 µL	DNase I,† 8 units/µL	–20°C
850 µL	20X DNase Buffer	–20°C
1.2 mL	DNase Inactivation Reagent	–20°C
1 mL	Formaldehyde Load Dye	–20°C

* Store Wash Solutions at room temp if they will be used within 1 month, otherwise store at 4°C. Warm to room temp before use if stored at 4°C.

† The DNase I should be stored in a non-frost-free freezer.

C. Required Materials Not Provided with the Kit

- 100% ethanol: ACS grade or better
- 2 mL polypropylene microfuge tubes capable of withstanding 16,000 x g (e.g. 2 mL RNase-free Microfuge Tubes, P/N AM12425)
- Blood collection tubes (recommended anticoagulant: potassium EDTA or sodium EDTA)
- Pipettors and RNase-free pipet tips
- Heat blocks or incubators at 75°C and 37°C
- Microcentrifuge capable of an RCF of 16,000 x g

D. Related Products

RNase*Zap*® Solution P/N AM9780, AM9782, AM9784	RNase Decontamination Solution. RNase*Zap* is simply sprayed, poured, or wiped onto surfaces to instantly inactivate RNases. Rinsing twice with distilled water will eliminate all traces of RNase and RNase*Zap*.
RNase-free Tubes & Tips See our product catalog	RNase-free tubes and tips are available in most commonly used sizes and styles. They are guaranteed RNase- and DNase-free. For more information, see our product catalog at www.invitrogen.com/ambion.
Electrophoresis Reagents See our product catalog	We offer gel loading solutions, agaroses, acrylamide solutions, powdered gel buffer mixes, nuclease-free water, and RNA and DNA molecular weight markers for electrophoresis. For available products, see our product catalog at www.invitrogen.com/ambion.
RETROscript® Kit P/N AM1710	First strand cDNA synthesis kit for RT-PCR. When purchased with SuperTaq™, this kit provides reagents, controls and protocols for reverse transcription and PCR. Both oligo(dT) and random primers for cDNA priming are included, as is RNase inhibitor.

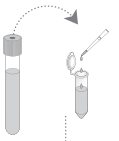
II. RiboPure-Blood Procedure

IMPORTANT

All centrifugation steps should be done at an RCF of ~16,000 xg—typically maximum speed on lab microcentrifuges. This corresponds to 13,200 rpm in an Eppendorf Model 5415D microcentrifuge.

Table 1. Procedure Overview

Collection and Stabilization of Blood



1. Collect blood samples using standard methods
2. (Optional) Add 300–500 µL blood to 1.3 mL RNAlater Solution

Cell Lysis and Initial RNA Isolation

1. Samples in RNAlater Solution: centrifuge and remove the supernatant
2. Lyse blood cells in 800 µL Lysis Solution and 50 µL Sodium Acetate Solution
3. Extract with 500 µL Acid-Phenol: Chloroform
4. Recover the aqueous phase in a fresh 2 mL tube
5. Add 600 µL of 100% ethanol to each sample

Final RNA Purification

1. Before you start
   a. Add 56 mL 100% ethanol to Wash Solution 2/3.
   b. Heat an aliquot of the Elution Solution to ~75°C in an RNase-free tube.
   c. Assemble and label the plastic ware.
2. Pass the sample through a Filter Cartridge ~700 µL at a time
3. Wash filter with 700 µL Wash Solution 1
4. Wash filter with 2 x 700 µL Wash Solution 2/3
5. Elute RNA with 2 x ~50 µL preheated Elution Solution

A. Blood Collection and (Optional) Stabilization of RNA in RNA*later* Solution

1. Collect blood samples using standard methods

Collect blood samples according to standard procedures in tubes containing anticoagulant. Potassium or sodium EDTA are the recommended anticoagulants, but heparin and citrate are also compatible with the procedure. Other anticoagulants have not been tested.

The standard protocol is designed for purification of RNA from 0.5 mL of whole blood. To process samples larger than 0.5 mL, either several purifications can be performed, or blood can be fractionated to enrich for white blood cells. For modifications to the procedure related to use of white blood cells (buffy coat), instead of whole blood, see *IV.A. Blood Fractionation and White Blood Cell Stabilization* starting on page 12.

2. (Optional) Add 300–500 µL blood to 1.3 mL RNA*later* Solution

a. Mix blood sample by gently inverting the collection tube several times.

b. Add 300–500 µL anticoagulated blood to 1.3 mL RNA*later* in a 2 mL microfuge tube (not included in the kit). Mix thoroughly by inverting the tube several times.

Once a sample is mixed with RNA*later*, it can be stored for up to 3 days at ambient temperature. Storing RNA*later* treated samples for longer periods at ambient temperature will result in a gradual decrease in RNA yield and quality.

Samples mixed with RNA*later* can also be stored at –20°C for long-term storage.

B. Cell Lysis and Initial RNA Purification

1. Samples in RNA*later* Solution: centrifuge and remove the supernatant

a. Centrifuge sample for 1 min in a microcentrifuge. The blood cells and plasma proteins will form a large brown or reddish-brown pellet which may smear upward along the side of the tube, and the supernatant may be pale pink, brown, or colorless (but it is often turbid).

b. Remove and discard the supernatant by aspiration or pouring.
- When aspirating the supernatant, be sure to thoroughly remove all of the fluid, including the portion directly above the cell pellet, which may be more turbid, and which may contain some white particulate matter. Note, this material is not the "buffy coat" fraction seen in untreated whole blood after centrifugation.
- If the supernatant is removed by pouring, tap the rim of the inverted tube gently against a paper towel to remove all residual fluid.
- Remove any fluid from inside the tube cap.

2. Lyse blood cells in 800 µL Lysis Solution and 50 µL Sodium Acetate Solution

a. If the blood sample was not stored in RNA*later* Solution, mix by gently inverting the collection tube several times.

b. Add 800 µL Lysis Solution and 50 µL Sodium Acetate Solution to 300–500 µL anticoagulated whole blood in a 2 mL microfuge tube, or to the cell pellet from RNA*later*-stabilized samples.

c. Vortex vigorously to lyse the blood cells. Invert the tube to be sure the solution is homogenous. Samples that were stabilized in RNA*later* will require more vigorous vortexing to resuspend and lyse the cells.

3. Extract with 500 µL Acid-Phenol: Chloroform

a. Withdraw 500 µL of Acid-Phenol:Chloroform from beneath the overlying layer of aqueous buffer, add it to the cell lysate, and shake vigorously or vortex for 30 sec.


NOTE

If addition of 500 µL of Acid-Phenol:Chloroform would cause the tube to be too full to permit adequate mixing, you can use as little as 250 µL of Acid-Phenol:Chloroform.

b. Store the mixture at room temp for 5 min.

c. Centrifuge at room temp for 1 min to separate the aqueous and organic phases. The aqueous phase may appear cloudy or clear after centrifugation.

4. Recover the aqueous phase in a fresh 2 mL tube

Transfer the aqueous (upper) phase containing the RNA to a new 2 mL tube (not provided in the kit). If samples were split for the Acid-Phenol:Chloroform extraction, collect the aqueous phases into a single tube.

Typically the aqueous phase volume is ~1–1.2 mL.

Avoid transferring the colored material from the organic (lower) phase, which contains heme and proteins. Discard the lower phase.


NOTE

If the aqueous phase volume is less than 800 µL, see section *III.B. Difficulty Recovering Aqueous Phase after Organic Extraction on page 10.*

5. Add 600 µL of 100% ethanol to each sample

To each tube of aqueous phase recovered after the Acid-Phenol: Chloroform extraction, add 600 µL (~one-half volume) of 100% ethanol, and vortex briefly but thoroughly.

If desired, the tube may then be centrifuged very briefly (~1 sec) to collect the fluid from around the lid of the tube.

C. Final RNA Purification

1. Before you start

a. Add 56 mL 100% ethanol to Wash Solution 2/3.

- Add 56 mL of 100% ethanol to the bottle labeled Wash Solution 2/3 Concentrate, and mix well.
- Store at room temp after the ethanol has been added if the Wash Solution will be used within 1 month. Store at 4°C for longer times, but warm to room temp before use.

b. Heat an aliquot of the Elution Solution to ~75°C in an RNase-free tube.

Typically each sample is eluted in ~100 µL, but additional Elution Solution may be heated to allow for evaporation.

c. Assemble and label the plastic ware.

- For each sample, place a Filter Cartridge into one of the Collection Tubes supplied in the kit. Label the lid of the resulting Filter Cartridge assembly.
- Label a second Collection Tube for each sample; these will be used to elute the RNA from the glass fiber filter.

2. Pass the sample through a Filter Cartridge ~700 µL at a time

a. Apply ~700 µL of the sample (aqueous phase mixed with ethanol from step II.B.5) to a Filter Cartridge assembly, and centrifuge for ~5–10 sec to pass the liquid through the filter. Discard the flow-through from the Collection Tube, and replace the Filter Cartridge into the same Collection Tube.

b. Load the next ~700 µL of sample onto the filter. Spin as before to pass the sample through the filter and discard the flow-through. Repeat to filter the remaining sample.

3. Wash filter with 700 µL Wash Solution 1

Apply 700 µL Wash Solution 1 to the Filter Cartridge assembly and centrifuge for ~5–10 sec to pass the solution through the filter. Discard the flow-through from the Collection Tube, and replace the Filter Cartridge into the same Collection Tube.

4. Wash filter with 2 x 700 µL Wash Solution 2/3

a. Apply 700 µL Wash Solution 2/3 (working solution mixed with ethanol) to the Filter Cartridge assembly and centrifuge for ~5–10 sec to pass the solution through the filter. Discard the flow-through from the Collection Tube, and replace the Filter Cartridge into the same Collection Tube.

b. Repeat with a second 700 µL aliquot of Wash Solution 2/3.

c. After discarding the flow-through from the last wash, replace the Filter Cartridge in the same Collection Tube and spin the assembly for 1 min to remove residual fluid from the filter.

5. Elute RNA with 2 x ~50 µL preheated Elution Solution

a. Transfer the Filter Cartridge into a labeled Collection Tube (provided with the kit). Apply ~50 µL Elution Solution (preheated to ~75°C) to the center of the filter, and close the cap. Leave the assembly at room temp for ~20 sec, then spin for ~20–30 sec at maximum speed to recover the RNA.

The exact volume of Elution Solution used to elute the RNA may be adjusted according to the desired RNA concentration. The RNA can be eluted in a lower volume to increase concentration; however, eluting in less than 2 x 50 µL may not result in thorough recovery of RNA.

b. Repeat with a second ~50 µL of Elution Solution, collecting the RNA into the same tube. Spin for 1 min to recover all of the Elution Solution in the Collection Tube.

c. Store the eluted RNA at –20°C or below.

D. (optional) DNase I Treatment

DNase I digestion removes contaminating genomic DNA from the eluted RNA.

1. Add 20X DNase Buffer and DNase I to the RNA

Add 1/20th volume 20X DNase Buffer and 1 µL DNase I (8 U/µL) to the eluted RNA and mix gently but thoroughly.

For example if the RNA was eluted in 100 µL Elution Solution, add 5 µL 20X DNase Buffer and 1 µL DNase I.

2. Incubate 30 min at 37°C

3. Add 20% volume of DNase Inactivation Reagent, mix thoroughly, and leave at room temp for 2 min

Use a volume of DNase Inactivation Reagent equal to 20% of the volume of RNA treated. For example if 100 µL of RNA is treated with DNase, add 20 µL of DNase Inactivation Reagent.

Vortex the tube briefly to thoroughly mix the DNase Inactivation reagent with the RNA, and store the sample at room temp for 2 min. Flick or vortex the tube once or twice during this period to resuspend the DNase Inactivation Reagent.

The DNase Inactivation Reagent is supplied as a slurry. Before removing it, vortex the tube vigorously to ensure that it is completely resuspended. To pipette the reagent, insert the pipet tip well below the surface and observe the aliquot withdrawn to ensure that it is mostly white, without a significant amount of clear fluid. If treating multiple samples, revortex the reagent as needed to resuspend the particles. The DNase Inactivation Reagent may become difficult to pipette after multiple uses due to depletion of fluid from the interstitial spaces. If this happens, add a volume of Elution Solution equal to approximately 10–20% of the bed volume of the remaining reagent and vortex thoroughly to recreate a pipettable slurry.

4. Pellet the DNase Inactivation Reagent and transfer the RNA to a new tube

Centrifuge the sample for 1 min in a microfuge to pellet the DNase Inactivation Reagent, then transfer the RNA solution to a new RNase-free tube (not supplied with the kit).

III. Troubleshooting

A. RNA Contains Residual Genomic DNA

Retreat the eluted RNA with DNase I (instructions are in section II.D)

Some samples have an unusually high white blood cell (WBC) count, and this results in a greater-than-normal DNA concentration. In other cases, for reasons that are not clear, there may be a relatively high DNA concentration in the absence of a high WBC count. Repeat the DNase treatment to remove residual genomic DNA.

Genomic DNA contamination is generally lower in blood samples that have been treated with RNAlater compared to samples in which the blood was mixed with Lysis Solution directly.

Use fewer PCR cycles and/or less RNA in the RT-PCR

Genomic DNA may be amplified in RT-PCR samples that are subjected to >30 PCR cycles and/or when an unusually large amount of RNA (>1 µg) is used in the RT-PCR.

B. Difficulty Recovering Aqueous Phase after Organic Extraction

Occasionally after the Acid-Phenol:Chloroform extraction step (step II.B.3 on page 6), the volume of the aqueous phase is less than ~1 mL, the interphase is unusually thick, and it is difficult to remove the aqueous phase without contaminating it with material from the organic phase. This may be caused by suboptimal mixing of the sample during the phenol:chloroform extraction step if the tube is too full to thoroughly mix the samples. This may also be seen in samples with an unusually high WBC content.

To recover the RNA from samples with less than ~0.8 mL of aqueous phase:

1. After the centrifugation step of the Acid-Phenol:Chloroform extraction, remove as much of the aqueous phase as possible to a new tube, and determine its approximate volume.
2. Add additional Lysis Solution to the organic phase to bring the total aqueous phase volume to 1.2 mL.
   For example, if 800 µL of aqueous phase was initially recovered in the acid phenol chloroform extraction, add 400 µL additional Lysis Solution to the tube with the organic phase.
3. Mix the Lysis Solution + organic phase by brief vigorous vortexing, then centrifuge for 1 min at maximum speed to separate the phases.
4. Remove the aqueous phase and pool it with the aqueous phase recovered initially.
5. Add one-half volume of 100% ethanol, mix well, and continue the procedure at step II.C.2 on page 7.

C. RNA Is Partially Degraded

The integrity of the RNA obtained with the RiboPure-Blood Kit may be compromised in "challenging" situations. One example of a challenging situation is processing a sample with a higher-than-expected WBC count, which could overload the capacity of the reagents to inactivate endogenous RNases. Other examples are when samples with high WBC counts are stored in RNA*later* for prolonged periods and/or at elevated temperature (>30°C) before processing, or using a suboptimal amount of RNA*later* to stabilize the blood sample. The following suggestions may improve RNA recovery/assay results in these situations:

1. Use a smaller volume of blood (300–400 µL instead of 500 µL)
2. Store samples stabilized in RNA*later* at –20°C instead of at room temperature, and/or isolate the RNA after a shorter time following stabilization in RNA*later*.
3. For RT-PCR assays, some degradation of RNA can be tolerated, especially for the small amplicons typically used in real-time RT-PCR assays. Therefore, it may be possible to use partially degraded RNA for RT-PCR experiments; for such experiments we recommend using PCR primers that amplify an amplicon of ~100–300 bp.

D. RNA Yield Is Lower than Expected

Lower than expected RNA yield may be due to improper sample processing (e.g. prolonged storage at elevated temperature, using blood samples larger than 500 µL), or to donor-specific variation in leukocyte count and/or in RNA content of the leukocytes.

IV. Additional Procedures

A. Blood Fractionation and White Blood Cell Stabilization

1. Dispense 1.2 mL RNA*later* into a 2 mL tube for each sample

For each sample, dispense 1.2 mL of RNA*later* into a 2 mL microfuge tube.

For samples expected to contain a higher-than-normal WBC (white blood cell) content (for example, leukemic blood samples) prepare 2 tubes of RNA*later* per sample.

2. Collect 2.5–10 mL blood samples

Collect 2.5–10 mL blood samples according to standard procedures in tubes containing anticoagulant (recommended anticoagulant is EDTA sodium or potassium salt).

3. Centrifuge samples at ~1500–2000 x g for 10–15 min at room temp

Fractionate the whole blood by centrifuging at 1500–2000 x g for 10–15 min at room temperature. This will separate the blood into an upper plasma layer, a lower red blood cell (RBC) layer, and a thin interface containing the WBCs (see Figure 1).

Although the suggested procedure is to fractionate the blood as soon as possible after collection, intact RNA has been recovered from EDTA-anticoagulated blood that was stored at room temp for 1 day before fractionation.

NOTE

In a typical clinical centrifuge 1500–2000 x g is ~3000–3400 rpm. For example, in an IEC CL2 table-top centrifuge using the 236 rotor and the 2092S carrier, centrifugation at 3000 rpm = 1560 x g, and 3400 rpm = 2000 x g.

4. Remove the plasma with a transfer pipet, being careful not to disturb the WBCs

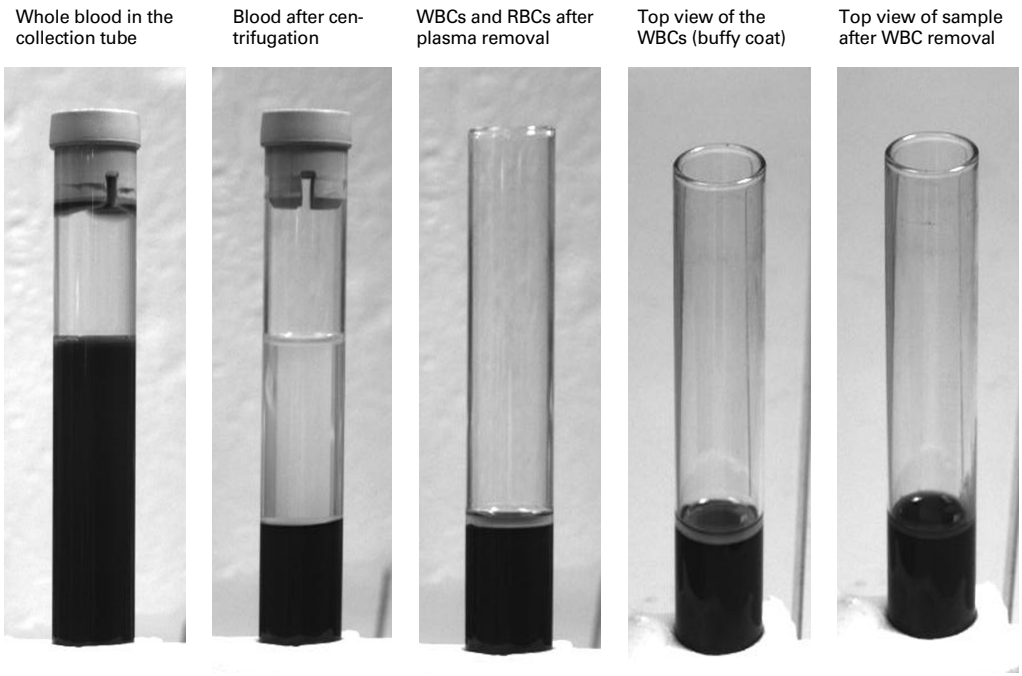
a. Use a disposable, plastic transfer pipet (e.g. Falcon Cat #357524) to aspirate off the plasma (upper layer) down to ~1 mm from the RBCs (see Figure 1). The plasma may be reserved for other applications (for example recovery of viral particles or for serological studies) or discarded.

When removing the plasma do not disturb the WBC layer, also called the buffy coat, which forms a thin film between the upper plasma layer and the lower layer of packed RBCs.

Samples with exceptionally high WBC counts will have a thicker buffy coat.

b. Expel all residual plasma from the transfer pipet before continuing.

Figure 1. Appearance of Blood Samples during Recovery of WBCs



5. Recover the WBCs in ≤ 0.5 mL by aspiration

Use the same transfer pipet to carefully aspirate the exposed WBC layer in a volume of about 0.5 mL or less. Aspirate slowly, using a circular motion, to pull all the visible buffy coat material into the transfer pipet. Some contamination of the WBCs with the underlying RBCs is expected.

Alternatively use a cytology brush to recover the WBCs.

6. Put the WBCs into a tube with 1.2 mL RNA*later* and mix well

a. Slowly expel the WBCs into a tube containing 1.2 mL of RNA*later*. Avoid introducing bubbles into the sample.
Recover any visible remaining WBCs using the same transfer pipet, being careful to keep the total volume <0.5 mL (WBCs in excess of ~0.5 mL will exceed the volume of the tube with RNA*later*). If the WBCs occupy more than 0.5 mL, split the sample into 2 aliquots using two tubes of RNA*later*.

IMPORTANT

At least 2 volumes of RNAlater are required to stabilize the RNA.

b. Aspirate up and down several times in the RNA*later* to rinse the pipet. Close the tube and mix the RNA*later* and WBCs thoroughly by vortexing or inversion.

7. Store the stabilized WBCs or continue with RNA isolation

This is a potential stopping point in the procedure; the stabilized WBCs can be stored at this point at ambient temperature (up to about 30°C) for up to about 5 days. Storage for longer than 5 days should be at –20°C. Continue RNA isolation from II.B starting on page 5.

B. Quantitation of RNA by UV Spectrophotometry

The concentration and purity of RNA can be determined by diluting an aliquot of the preparation (usually a 1:10 or 1:20 dilution) in TE (10 mM Tris-HCl pH 8, 1 mM EDTA), and reading the absorbance in a spectrophotometer at 260 nm and 280 nm. Be sure to zero the spectrophotometer with the TE used for sample dilution.

1. Concentration

An A_{260} of 1, in spectrophotometer with a 1 cm path length, is equivalent to 40 µg RNA/mL.

The concentration (µg/mL) of RNA is calculated by multiplying the A_{260} x dilution factor x 40 µg/mL.

Following is a typical example:

RNA is isolated from 500 µL whole blood and eluted in 100 µL Elution Solution.
5 µL of the prep is diluted 1:20 into 95 µL of TE and read in a microcuvette
A_{260} = 0.024
RNA concentration = 0.024 x 20 x 40 µg/mL = 19.1 µg/mL
RNA yield = (19.1 µg/mL) x 0.1 mL = 1.91 µg
RNA yield per mL of blood = 3.82 µg

Be aware that contaminating genomic DNA will lead to an overestimation of yield, since all nucleic acids absorb at 260 nm.

2. Purity

The ratio of A_{260} to A_{280} values is a measure of RNA purity, and it should fall in the range of ~1.9 to 2.2. In the above example, the A_{280} value was 0.012, so the A_{260}:A_{280} is 0.024/0.012 = 2.0. This indicates that the RNA is not contaminated with significant amounts of protein or phenol.

C. Assessing RNA Quality on Denaturing Agarose Gel

For more information, go to www.invitrogen.com/site/us/en/home/support/technical-support.html.

Mix sample RNA with one half volume of Formaldehyde Load Dye (included with kit) and heat for 5 min at 65–70°C. Typically 15–20 µL of RNA is an appropriate amount for gel analysis when the RNA is eluted in a volume of 100 µL. Analyze the sample on a denaturing agarose gel. Intact RNA will have sharp bands consisting of 28S and 18S ribosomal RNA, migrating between the two tracking dye bands. Partially degraded RNA will show smeared bands and/or a reduction in the ratio of the 28S:18S rRNA band.

V. Appendix

A. Quality Control

Functional testing

RNA is isolated from whole blood following the instructions in this booklet to demonstrate that ≥3 µg RNA can be obtained per mL of blood.

Nuclease testing

Relevant kit components are tested in the following nuclease assays:

RNase activity
Meets or exceeds specification when a sample is incubated with labeled RNA and analyzed by PAGE.

Nonspecific endonuclease activity
Meets or exceeds specification when a sample is incubated with supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.

Exonuclease activity
Meets or exceeds specification when a sample is incubated with labeled double-stranded DNA, followed by PAGE analysis.

Protease testing

Meets or exceeds specification when a sample is incubated with protease substrate and analyzed by fluorescence.

B. Safety Information

WARNING

GENERAL SAFETY. *Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.*

- *Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.*
- *Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.*

1. Chemical safety

WARNING

GENERAL CHEMICAL HANDLING. *To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:*

- *Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.*
- *Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).*
- *Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).*
- *Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.*
- *Handle chemical wastes in a fume hood.*
- *Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)*
- *After emptying a waste container, seal it with the cap provided.*
- *Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.*
- *Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.*
- ***IMPORTANT!*** *Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.*

2. Biological hazard safety

WARNING

Potential Biohazard. *Depending on the samples used on the instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.*

WARNING

BIOHAZARD. *Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:*

In the U.S.:

- *U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: www.cdc.gov/biosafety*
- *Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html*
- *Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.*
- *Additional information about biohazard guidelines is available at: www.cdc.gov*

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/

VI. Documentation and Support

A. Obtaining SDSs

Safety Data Sheets (SDSs) are available from:

www.invitrogen.com/sds

or

www.appliedbiosystems.com/sds

Note: For the SDSs of chemicals not distributed by Life Technologies, **contact the chemical manufacturer.**

B. Obtaining support

For the latest services and support information for all locations, go to:

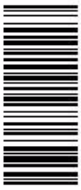
www.invitrogen.com

or

www.appliedbiosystems.com

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches



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