Applied Biosystems

Total RNA Purification from Whole Blood

- **Isolate total RNA directly from animal and human whole blood without any sample pre-treatment using any common anticoagulant**
- **96-well format purification of whole blood or peripheral blood mononuclear cells (PBMCs) gives you higher throughput than ever before—96 samples in less than one hour**
- **Novel chemistry combined with unique hardware and disposables gives you the highest quality and yield of total RNA available**

Abstract

Applied Biosystems has developed new techniques that produce the highest-quality total RNA from whole blood or isolated blood cells from various animal species and a variety of anticoagulants, including heparin. The chemistry allows you to isolate highly pure, highly stable total RNA directly from whole blood, without sample pre-treatments such as proteinase K digestion or red blood cell lysis.

Introduction

Many of today's gene expression studies that use real-time PCR or microarrays require high-quality RNA purified from whole blood. Study RNA must be highly pure, because accuracy and sensitivity are critical for interpretation of results. Automation of RNA purification from whole blood is a formidable challenge. Blood contains high levels of protein, genomic DNA (gDNA), and RNases. The presence of high levels of RNases and multiple PCR inhibitors (heme, immunoglobulin G, and lactoferrin) plague existing extraction procedures and downstream assays. Blood samples collected with the anticoagulant heparin are also known to inhibit PCR. Contaminating gDNA may affect the accuracy of RT-PCR, and other associated procedures. Automated preparation of RNA from blood must deal with these factors, and provide material that is highly pure, free from contaminating gDNA, and inhibitors of PCR or reverse transcription.

Applied Biosystems has developed instrument and reagent systems to enable purification of 96 samples of total RNA from whole blood. This purification procedure generates total RNA free of PCR inhibitors, RNases, and contaminating gDNA. All three common types of blood anticoagulants—citrate, EDTA, and heparin—are compatible with the RNA purification procedure. The lysis reagent has potent RNase inhibition properties and the protocol utilizes all other existing Applied Biosystems total RNA reagents and consumables.

Many protocols for isolation of total RNA from blood incorporate a leukocyte isolation process using either centrifugation or selective red blood cell (RBC) lysis. Bulk removal of RBCs and plasma components greatly facilitates the isolation of pure, intact RNA. However, these processes often add cost or are time and labor intensive. Additionally, front-end processing of the above types do not neutralize biohazardous agents in the samples.

Total RNA Isolation From Whole Fresh Blood From a Variety of Animal Species

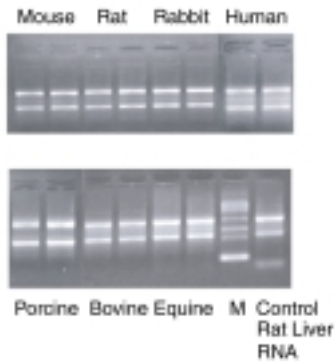


Figure 1. To one volume of whole, fresh (never frozen) blood from mouse, rat, rabbit, human, pig, cow, and horse, one volume of phosphate-buffered saline and two volumes of 2X Total RNA Lysis Reagent (P/N 4305895) was added. Following thorough mixing, a minimum of 1 mL per well of each blood lysate was processed for total RNA on the ABI PRISM 6100 Nucleic Acid PrepStation. 2 µg of eluted RNA was analyzed in duplicate on a 1.2% agarose gel. The lane designated "M" is a molecular weight marker.

Blocking RNase activity in blood lysates is difficult because RNase activity in blood is much higher than in other tissues. The direct lysis of whole blood is easier, faster, and safer. Applied Biosystems total RNA isolation process lyses blood directly, providing fast, high-throughput purification.

You can purify total RNA from whole blood on the ABI PRISM™ 6100 Nucleic Acid PrepStation or ABI PRISM™ 6700 Automated Nucleic Acid Workstation. The RNA purification chemistry successfully isolates pure total RNA from human blood within a concentration range of

2–8 µg per mL of whole blood. RNA can be isolated from several animal species as demonstrated in Figures 1 and 3. (Note that RNA concentration varies depending on the animal species or the health of the patient.)

You can process PBMC samples using the existing protocol, provided the samples are isolated from fresh blood and not frozen before cell lysis.

Blood Sample Types

- Citrate, EDTA, or heparin as anticoagulant
- PBMC (peripheral blood mononuclear cell)
- Mouse, rat, rabbit, porcine, bovine, and equine

Removing Genomic DNA Contamination

One of the key measures of total RNA purity is the level of genomic DNA (gDNA) contamination. Limited transcript sequence knowledge or design constraints of the 5' nuclease assay, TaqMan® probe, and primer target sets makes eliminating possible detection of gDNA (rather than messenger RNA in RT-PCR amplification reactions) difficult. This potentially serious experimental limitation can cause ambiguous expression results. Therefore, you should make every effort to ensure that total RNA analyzed is essentially gDNA free, with as close to 0% gDNA by weight as possible.

In a simple, 15-minute incubation step during the total RNA isolation procedure, AbsoluteRNA Wash Solution (P/N 4305545) ensures the total RNA isolated has <0.002% of gDNA (as measured by the 5' nuclease-based TaqMan® assay). In addition, the reagent removes the potent PCR inhibitor heparin from heparin anticoagulated blood samples.

Stability of RNA Isolated Using Applied Biosystems Total RNA Chemistry

RNA isolated using this chemistry is both highly pure and highly stable. $A_{260/280}$ ratios of isolated RNA are routinely > 1.9. This means the RNA is free from the majority of contaminating proteins including RNases.

The perceived lack of stability of RNA at room temperature comes from the presence of such proteins. Highly pure RNA, free from such proteins and metal ions, is stable for a number of hours (Figure 2). This experiment shows gel images of human-blood-derived total RNA after heating for three hours at 37°C, and standing at room temperature overnight.

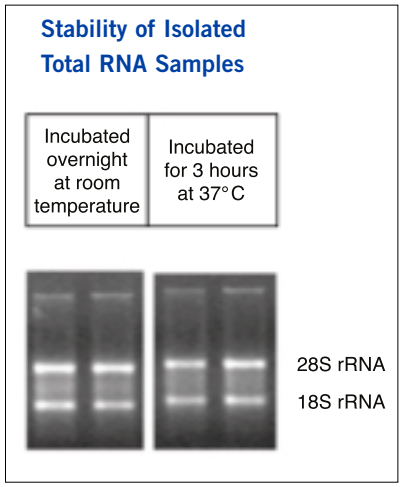


Figure 2. This figure shows approximately 2 µg of RNA per lane run in duplicate on a 1.2% agarose gel. 4 x 500 mL blood/PBS/lysis solution (0.5 mL whole human blood total) was processed. The data indicates that total RNA obtained using Applied Biosystems chemistry is highly stable and highly pure.

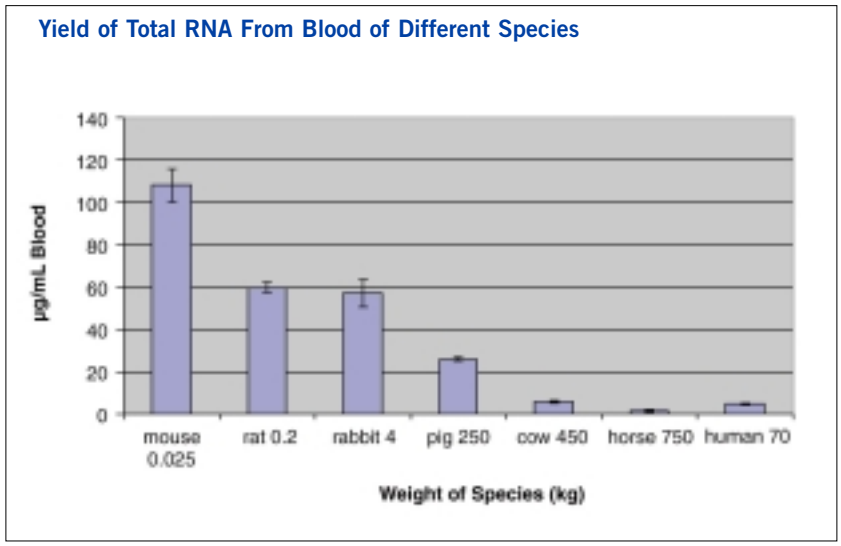
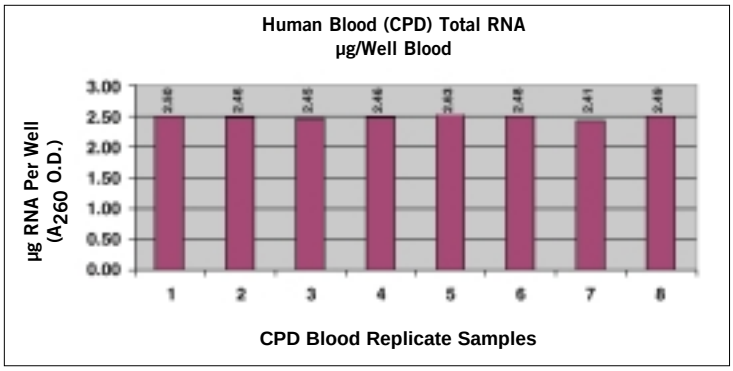
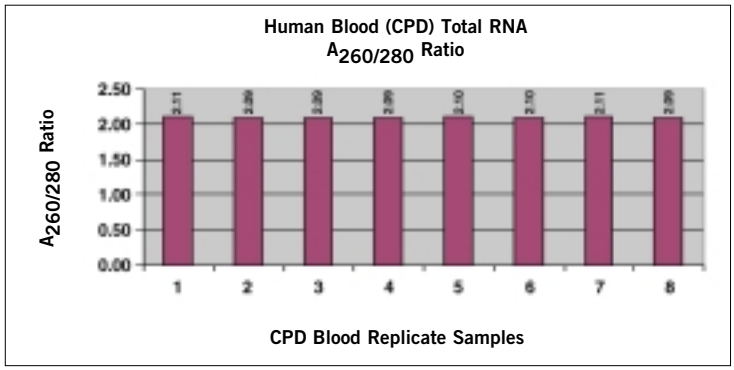
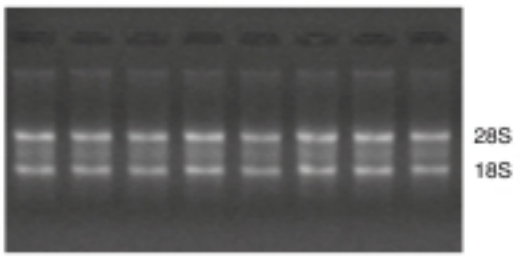


Figure 3. Yields of total RNA obtained from a variety of species, including human, expressed as µg of RNA isolated per mL of whole blood.

Yield of Total RNA From Human Whole Blood



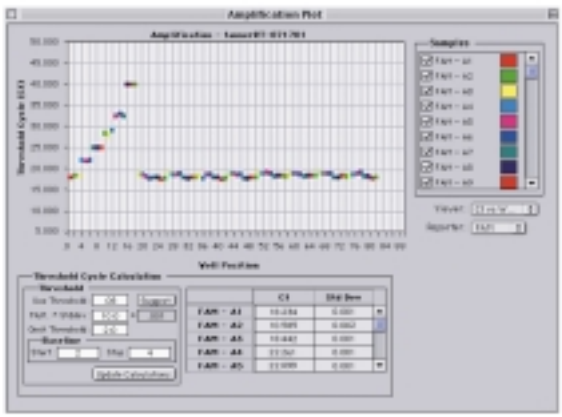




Total RNA from Human Whole Blood

Figure 4. From 500 µL whole blood (citrate, phosphate, and dextrose anticoagulated), 2 mL of lysate was generated and purified on the ABI PRISM 6100 PrepStation. This yielded approximately 2.5 µg RNA per well with an average $A_{260/280}$ ratio of 2.1. Agarose gel image of the RNA shows intact and undegraded 18 and 28S bands.

Human Cyclophilin Quantitative RT-PCR



0.5 mL Whole Blood (1:50 dilution)

Figure 5. Human total RNA was isolated from 96, 0.5 mL whole human blood samples using the ABI PRISM 6100 Nucleic Acid PrepStation and incorporating an AbsoluteRNA Wash Solution, gDNA removal step. The total RNA was diluted 1:50 and amplified in a 5' nuclease-based, one-step RT-PCR assay for the cyclophilin amplicon on the ABI PRISM® 7700 Sequence Detection System. The amplification plot of C_T versus well position indicates that, for the 78 samples analyzed, the yield of RNA was highly reproducible well-to-well.

Wells 1–15: Human control RNA standard curve 5 ng/µL to 0.0005 ng/µL

Wells 16–19: No template controls

Wells 20–96: Amplification reaction

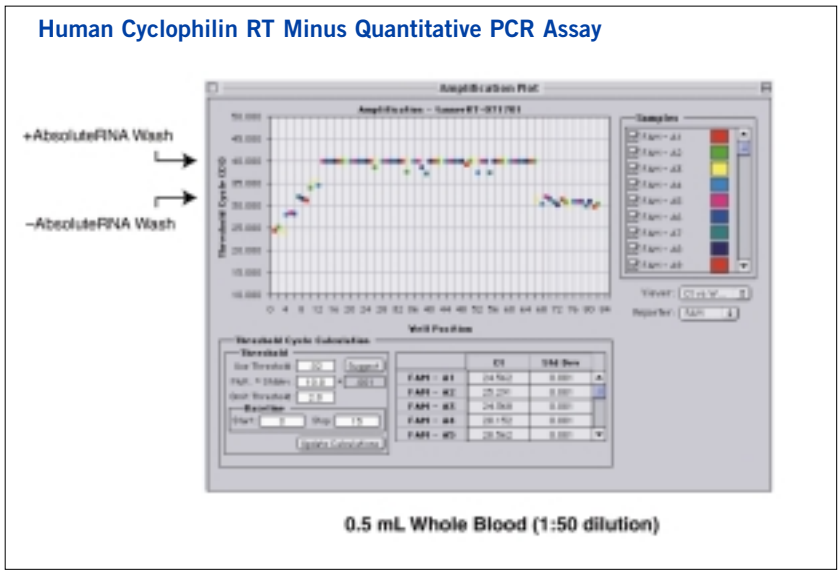


Figure 6. Same assay as outlined in Figure 5, but the amplification reaction was performed without reverse transcriptase (i.e., only contaminating gDNA carried through the total RNA isolation step was amplified).

Wells 1–12: Human control gDNA standard curve

Wells 13–16: No template controls

Wells 17–65: Samples treated with AbsoluteRNA Wash Solution and amplified for contaminating gDNA

Wells 66–96: Samples not treated with AbsoluteRNA Wash Solution and amplified for contaminating gDNA

Methods

I. Isolation of Total RNA from Whole Blood Using the ABI PRISM 6100 Nucleic Acid PrepStation

Summary of the ABI PRISM 6100 PrepStation RNABlood-gDNA Protocol

Step	Description	Position	Incubate	Vacuum	Vacuum time/secs	Volume/µL
1.	Pre-wet all wells/ Wash Solution 1	Waste		0		40
2.	Load samples	Waste		80%	180	125–750*
3.	Wash Solution1	Waste		80%	180	650
4.	Wash Solution 2	Waste		80%	180	650
5.	AbsoluteRNA Wash Solution	Waste	15 mins	0**	0	50
6.	Wash Solution 2	Waste	5 mins	80%	180	600
7.	Wash Solution 2	Waste		80%	180	650
8.	Pre-elution vacuum	Waste		90%	300	
9.	Touch off	Waste		–		
10.	Elution Solution	Collection		20%	120	150
11.	Touch off***	Collection		–		

* 125–750 µL of whole blood, equivalent to 500–3,000 µL of lysate. Add 500 µL aliquots and then operate vacuum. Repeat until all lysate is added.

** Caution. Do not operate the 6100 PrepStation's vacuum after addition of AbsoluteRNA Wash Solution until you have completed the incubation following addition of Wash Solution 2. Operation of the vacuum prior to incubation will remove the reagent from contact with the purification tray membrane and increase the amount of gDNA present in the RNA sample.

*** Total protocol time: approximately 1.5 hours.

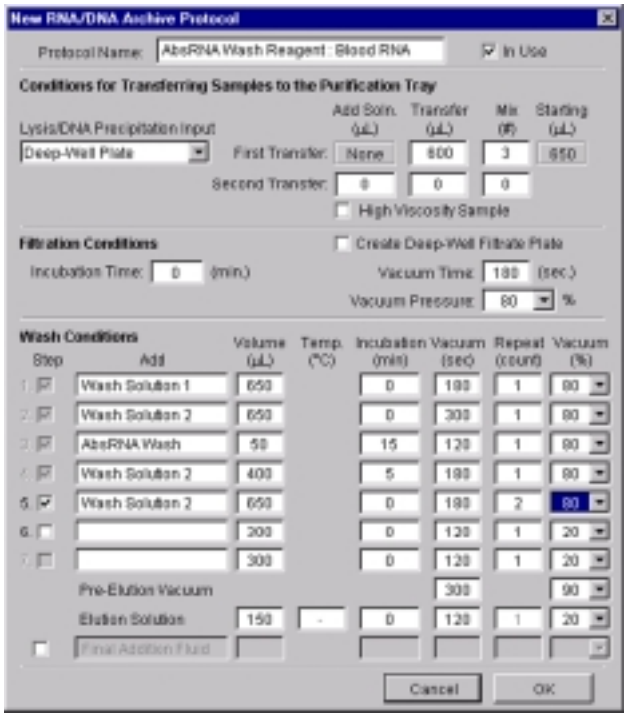
Summary of the ABI PRISM 6100 PrepStation RNABlood Protocol

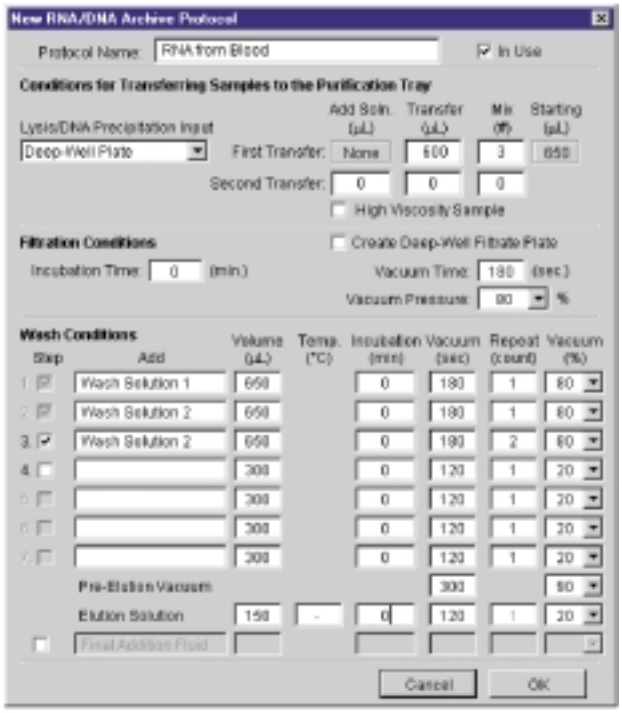
Step	Description	Position	Incubate	Vacuum	Vacuum time/secs	Volume/µL
1.	Load samples	Waste		80%	180	125–750*
2.	Wash Solution 1	Waste		80%	180	650
3.	Wash Solution 2	Waste		80%	180	650
4.	Wash Solution 2	Waste		80%	180	650
5.	Wash Solution 2	Waste		80%	180	650
6.	Pre-elution vacuum	Waste		90%	300	
7.	Touch off	Waste		–		
8.	Elution Solution	Collection		20%	120	150
9.	Touch off**	Collection		–		

* 125–750 µL of whole blood, equivalent to 500–3,000 µL of lysate

** Total protocol time: approximately 1 hour.

II. Isolation of Total RNA From Whole Blood Using the ABI PRISM 6700 Automated Nucleic Acid Workstation





Ordering Information

Description	Quantity	P/N
ABI Prism™ 6100 Nucleic Acid PrepStation		6100-01
ABI Prism™ 6700 Automated Nucleic Acid Workstation		6700-01
2X Total RNA Lysis Reagent	250 mL	4305895
Wash Solution 1	1,000 mL	4305891
Wash Solution 2	1,000 mL	4306890
Elution Solution	1,000 mL	4305893
AbsoluteRNA Wash Solution	10 mL	4305545
Total RNA Purification Tray	10 Per Box	4305673
Deep Well Plates	10 Per Box	4306841
Splash Guard	20 Per Box	4311758
96-Well Microplate	20 Per Box	4306737

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Printed in the USA, 2/2002, JPI
Publication 117AP03-02

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