ABIOpure™ Extraction

ABIOpure™ Total RNA (version 2.0)

Blood Extraction Handbook

Cat No: M541RP50-B

FOR RESEARCH USE ONLY

AllianceBio

Table of Contents

Kit Components

Component	M541RP50-B	Storage Temp
No. of preps	50	
RBC Lysis	50 ml	4°C
Buffer RB1	60 ml	Room Temperature
Buffer RBW	30 ml	Room Temperature
Buffer RBW	30 ml	Room Temperature
RNase-free water	15 ml	Room Temperature
ABIOpure™ filter (yellow) (with collection tube)	50 pcs	Room Temperature
ABIOpure™ column type W (blue ring) (with collection tube)	50 pcs	Room Temperature
1.5 ml collection tube	50 pcs	Room Temperature

Precautions

RBC Lysis Buffer contains phenol which is poisonous and quinidine salt which is an irritant. Care should be taken during handling. Always wear a lab coat, disposable gloves, protective goggles and follow standard safety precautions. In case of contact, wash immediately with plenty at room temperature water and seek medical advice.

Handle and dispose of all biological samples as if they were able to transmit infective agents. Avoid direct contact with the biological samples. Avoid producing spills or aerosol. Any material coming in contact with the biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite or autoclaved for one hour at 121°C before disposal.

Never pipette solutions by mouth! Handle and dispose of all reagents and all materials used to carry out the assay as if they were able to transmit infective

agents. Avoid direct contact with the reagents. Avoid producing spills or aerosol. Waste must be handled and disposed of according to adequate safety measures. Disposable combustible material must be incinerated

Stability and Storage

ABIOpure™ Total RNA Blood Extraction kit, except the RBC Lysis Buffer, should be stored at room temperature.
RBC Lysis should be stored 4°C for optimal performance.
All components are stable for 1 year.

General Description

ABIOpure™ Total RNA Blood Extraction kit is designed for the purification of total RNA from fresh mammalian whole blood. This kit utilizes cell lysis and purification based on glassfiber membrane technology.
Whole blood sample is homogenized and lysed in the RBC Lysis Buffer, a monophasic solution containing phenol and guanidium salt, which rapidly lyses cells and inactivates the nucleases. In conventional methods, the erythrocytes of mammalian blood, which do not contain nuclei (nor RNA), should be removed by pretreatment such as osmotic lysis. This type of pretreatment increases the experiment time and the possibility of RNA-breakage, followed by decline of RNA quality.
The ABIOpure™ Total RNA Blood Extraction kit does not need additional pretreatment as the blood is lysed in one step. Then addition of chloroform effects a separation of the lysate into aqueous and organic phases. After phase-separating, DNA and proteins remain in the interphase and the organic phase respectively, and the released RNA exists in the aqueous phase.
The aqueous phase is picked and applied to an ABIOpure™ filter to eliminate small amounts of contaminated DNA and other blood contaminants. The passed-through is mixed with Buffer RB1, RNA binding buffer, and then the mixture is applied to a mini spin column. After a series of washing with Buffers RBW and RNW, pure RNA can be eluted by RNase-free water.

The entire procedure can be completed within 30 min and the purified RNA is ready for use in the isolation of Poly A+ RNA, Northern blotting, dot blotting, in vitro translation, cloning, RT-PCR, PRA and other analytical procedures.

Limitations

The ABIOpure™ Total RNA Blood Extraction kit is intended for Research Use Only applications.

Quality Control

All components of the ABIOpure™ Total RNA Blood Extraction kit are manufactured in a clean environment which is monitored periodically. To ensure product consistency and quality, the quality certification process is carried out on each lot of product.

Technical Support

Technical Support

If you need assistance, have any question or suggestion or if you experience any difficulties using ABIOpure™ extraction kits, please feel free to contact our technical support team at support@alliancebio.com.

Features

Format: Spin Column
Maximum amount of starting sample: 0.25 ml
Minimum amount of starting sample: 0.10 ml
Maximum loading volume: 700 µl
Minimum elution volume: 30 µl
Maximum binding capacity: 100 µg
Typical yield: 3 µg
Operation time: 30 minutes

Samples

Up to 250 µl of mammalian whole blood.
Collect blood samples in EDTA-Na_2 treated collection tubes (or other anticoagulant mixture that does not affect your later application).
Start the extraction procedure within 4 hours after collection or store at 4°C for maximum 12 hours before extraction. To ensure that the RNA extract contains enough distribution of mRNAs, avoid storage of samples more than few hours after isolation.
For long-term storage, it is recommended to store the sample lysate at -70°C (after proper mixing and homogenization with Buffer RB and β-mercaptoethanol).

Materials required but not provided

- Laminar flow hood
- Pipette set (10 µl, 100 µl and 1000 µl)
- RNA-free pipet tips
- Sterile 1.5 microcentrifuge tubes (nuclease-free)
- Microcentrifuge for centrifugation at 4^0C and at room temperature
- Chloroform or I-bromo-3-chloropropane (BCP)
- Suitable protection (ex. lab coat, disposable gloves, goggles, etc.)

Mammalian Blood Protocol

1.	**Add 750 µl RBC Lysis Buffer in a 1.5 ml microcentrifuge tube (not provided).**
2.	**Add 250 µl blood sample to the 1. 5 ml microcentrifuge tube and vortex vigorously.**

	If sample volume is 100 µl, sample should be adjusted to 250 µl with PBS or RNase-free water. Be sure to confirm the applicable minimum volume, which is 100 µl.
3.	**Incubate 2 minutes at room temperature.** This step allows leukocytes to be completely collapsed.
4.	**Add 0.2 ml chloroform. Shake vigorously for 15 seconds and let stand 2 minutes at room temperature.** Alternatively, 0.1 ml BCP (I-bromo-3-chloropropane) can be used in place of chloroform.
5.	**Centrifuge at 12,000 x g for 15 minutes at 4^0C.** The mixture will be separated into 3 phases: a lower layer, an interphase, and a colorless upper aqueous layer. The upper aqueous volume is approx. 450 µl. Centrifugation at > 8^0C may cause some DNA to partition in the aqueous phase.
6.	**Transfer the aqueous phase (approx. 450 µl) to the ABIOpure™ filter (yellow).** Small amount of DNA and other blood contaminants are eliminated by the ABIOpure™ filter.
7.	**Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.**
8.	**Add 2X volume (usually 900 µl) of Buffer RB1 to the collection tube including the passed-through, and mix well by pipetting.**

	Do not centrifuge at this step.
9.	**Transfer up to 700 µl of mixture to an ABIOpure™ column (blue ring).**
10.	**Centrifuge at > 10,000 x g for 30 seconds at room temperature.** **Discard the passed-through and reinsert the mini spin column back into the same tube.**
11.	**Repeat steps 9 ~ 10 using the remainder of the sample.**
12.	**Add 500 µl of Buffer RBW to the mini spin column.**
13.	**Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.** **Discard the passed-through and reinsert the mini spin column back into the same tube.**
14.	**Add 500 µl of Buffer RNW to the mini spin column.**
15.	**Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.** **Discard the passed-through and reinsert the mini spin column back into the same tube.**
16.	**Centrifuge at ≥ 10,000 x g for an additional 1 minute at room temperature to remove residual wash buffer** **Transfer the mini spin column to a new 1.5 ml microcentrifuge tube (provided).**

	Residual ethanol may interfere with downstream reactions. Care must be taken at this step for eliminating the carryover of Buffer RNW.
17.	**Add 50 µl of RNase-free water to the center of the membrane in the mini spin column.** To increase the RNA concentration, reduce the elution volume at least 30 µl.
18.	**Centrifuge at 10,000 x g for an additional 1 minute at room temperature.** Purified RNA can be stored at 4°C for immediate analysis and can be stored at -70°C for long term storage. The purified RNA is free of DNA and proteins, and A260/A280 will be between 1.8 and 2.2.

Troubleshooting

Problem	Possible Causes / Suggested Solutions
Low yield of RNA	**Poor quality of blood sample** Process the fresh blood sample immediately.
	Sample not completely lysed Vortex the sample vigorously. Be sure to incubate for 2 minutes at room temperature after lysis step.
	Too much or too less blood sample

	It may cause an inefficient lysis effect. Use the appropriate sample volume from 100 µl to 250 µl.
	Some aqueous phase left Perform 2nd extraction with the remaining aqueous phase.
	Incorrect elution conditions Add RNase-free water to the center of the mini spin column membrane.
Degradation of RNA	**Sample manipulated too much before the addition of RBC Lysing Buffer** Process the blood sample immediately after harvest
	RNA still bound to the RB Column membrane Store blood sample at -70^{0}C (not recommended). As storage time increases, RNA condition will deteriorate.
	Reagent or disposable is not RNase-free Make sure to use only RNase-free products
	Inappropriate handling of starting material Ensure handling and storage of samples according to the protocol
	RNase contamination Avoid working on areas where DNA preparation is performed with the presence of RNase.
Low A260/280 (<1.6)	**Aqueous phase was contaminated with the phenol phase**

	Avoid carryover when transferring the aqueous phase to ABIOpure™ filter.
	Sample not completely lysed with RBC Lysing Buffer Use 750 µl RBC Lysing Buffer for up to 250 µl blood sample. Be sure to incubate sample for 2 minutes at room temperature after lysis step.
Hi DNA contamination	**No treatment with DNase** Perform one or both optional steps of DNA Residue Degradation.

Ordering Information

Product Name	Cat. No.	# of Preps
ABIOpure™ Total DNA Blood/Tissue/Cell	M501DP50	100 preps
ABIOpure™ Total RNA Cell-Free Fluids	M541RP50-A	50 preps
ABIOpure™ Total RNA Blood	M541RP50-B	50 preps
ABIOpure™ Viral DNA/RNA	M561VT50	50 preps

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