ABIOpure™ Extraction

ABIOpure™ Total RNA
(version 2.0)

Cell-Free Extraction Handbook

Cat No: M541RP50-A

FOR RESEARCH USE ONLY

AllianceBio

Table of Contents

Kit Components

Component	M541RP50-A	Storage Temp
No. of preps	50	
Buffer NVL	16 ml	Room Temperature
Buffer RB1*	5 ml	Room Temperature
Buffer RBW*	13 ml	Room Temperature
Buffer RNW*	6 ml	Room Temperature
RNase-free water	15 ml	Room Temperature
Carrier RNA**	370 µg	Room Temperature
ABIOpure™ column type S (with collection tube)	50 pcs	2-8°C
1.5 ml microcentrifuge tube	50 pcs	Room Temperature

*Before first use, add absolute ethanol (ACS grade or better) into buffers RB1, RBW, and RNW as indicated on the bottle

**Refer to page 6 for Carrier RNA

Precautions

Buffer NVL, RB1 and RBW contain irritant which is harmful when in contact with skin or eyes, or when inhaled or swallowed. 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 °121C 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 Cell-Free Extraction kit should be stored at room temperature. After reconstitution of Carrier RNA with nuclease-free water, it should be stored in aliquots at -20°C for conservation of activity or immediately used for experiments.

Under cool ambient conditions, a precipitate can be formed in Buffer NVL. In such a case, heat the bottle above 37°C to dissolve completely.

General Description

ABIOpure™ Total RNA Cell-Free Extraction kit provides a convenient method for isolation of RNA and DNA from cell-free fluid, cell-culture supernatant, plasma, serum, urine and virus-infected samples.

ABIOpure™ Total RNA Cell-Free procedure employ the glassfiber membrane technology for the fastest and the most convenient high purity RNA and DNA isolation, instead of conventional alcohol precipitation of phenol/chloroform extraction.

ABIOpure™ Total RNA Cell-Free buffer system provides the effective binding condition of RNA and DNA to glassfiber member and the impurities on the membrane are washed away by two different wash buffers. Then pure RNA and DNA are eluted by nuclease-free water. The procedure takes only 15 minutes and the purified nucleic acid is suitable for RT-PCR, PRA and other analytical procedures.

ABIOpure™ Total RNA Cell-Free should be performed at room temperature. The purified nucleic acid should be treated with care as RNA is very sensitive to contaminants, such as RNases, often found on general labware and in dust. To

ensure RNA stability, it is recommended to store at 4°C for immediate analysis or to freeze at -70°C for long term storage.

Limitations

The ABIOpure™ Total RNA Cell-Free Extraction kit is intended for Research Use Only applications.

Quality Control

All components of the ABIOpure™ Total RNA Cell-Free 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

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
Maximum amount of starting sample: 100 µl / prep
Maximum loading volume: 750 µl
Minimum elution volume: 20 µl
Operation time: 15 minutes

Samples

Starting material, such as plasm or serum, should be stored at -70°C in aliquots for long term storage. Repeated freezing and thawing of frozen plasm or serum leads to protein precipitation, causing reduced viral titers and subsequently decreased yields of the isolated viral nucleic acid. In addition, protein precipitant will cause clogging of the spin column.

ABIOpure™ Total RNA Cell-Free Extraction kit is designed to extract total nucleic acids from samples including virus and host cell. The use of cell-free body fluids is recommended for isolation of the viral nucleic acid and the extraction efficiency can vary depending on the type of virus and sample media.
Provided Carrier RNA can help to improve the binding of viral nuclei acids to the spin column especially in the case of very few target nucleic acids in the samples and protect target nucleic acids from the chance of degradation due to residual RNase activity.

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 room temperature
- Suitable protection (ex. lab coat, disposable gloves, goggles, etc.)

Carrier RNA

This kit is provided with Carrier RNA which can be added at the lysis step, if required. Carrier RNA enhances binding of nucleic and to the spin column membrane, especially if there are very few target molecules in the sample.
For purification of nucleic acid from very small amounts of sample, we recommend adding Carrier RNA at the lysis step. To obtain a solution of 1 µg/µl, add 370 µl of nuclease-free water to the tube containing 370 µg lyophilized Carrier RNA. Dissolve the carrier RNA thoroughly, divide into conveniently sized aliquots, and store at -20°C. Do not freeze-thaw the aliquots of Carrier RNA more than 3 times. For one preparation, 7 µl of dissolved Carrier RNA is required.

Protocol

Before first use, add absolute ethanol (ACS grade or better) into buffers RB1, RBW, and RNW as indicated on the bottle

1.	**Add 300 µl of Buffer NVL and 7 µl of Carrier RNA into a tube.**
2.	**Transfer up to 100 µl of serum sample into the tube** If the sample volume is less than 100 µl, adjust the volume to 100 µl with PBS. In case of large sample volume, increase the amount of Buffer NVL and Carrier RNA proportionally.
3.	**Mix thoroughly by vortexing for 10 seconds.** For proper lysis, the complete mix of sample and Buffer NVL is essential.
4.	**Incubate the mixture for 10 minutes at room temperature.**
5.	**Add 350 µl of Buffer RB1 to the mixture and mix thoroughly by vortexing for 10 seconds.** The volume of Buffer RB1 can be adjusted in proportion to the volume of lysate. Do not centrifuge at this step. Nucleic acids can be precipitated through centrifugation.

6.	**Transfer up to 750 µl of the mixture to a spin column (column type micro S, white).**
7.	**Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.** Discard the pass-through and re-insert the spin column back into the same tube. If the sample exceeds 750 µl, repeat step 6-7 with the remainder of the sample.
8.	**Add 500 µl of Buffer RBW to the spin column.**
9.	**Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.** Discard the pass-through and re-insert the spin column back into the same tube.
10.	**Add 500 µl of Buffer RNW to the spin column.**
11.	**Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.** Discard the pass-through and re-insert the spin column back into the same tube.
12.	**Centrifuge at full speed for an additional 1 minute at room temperature to remove residual wash buffer.** **Transfer the spin column to a new 1.5 ml microcentrifuge tube (provided).** Residual alcohol may interfere with downstream reactions. Care must be taken at this step for eliminating the carryover of the Buffer RNW.

13.	**Add 25-50 µl of nuclease-free water to the center of the membrane in the spin column.** **Let it stand for one minute.**
14.	**Centrifuge at 10,000 x g for 1 minute at room temperature.** Purified nucleic acids can be stored at 4˚C for immediate analysis and can be stored at -70˚C for long term storage.

Troubleshooting

Problem	Possible Causes / Suggested Solutions
Low yield	**Poor quality of starting material** Repeated freezing and thawing should be avoided.
	Low concentration of virus in the sample. Use more sample. Concentrate the sample volume to 300 µl using a microconcentrator.
	Sample not homogenized completely. Be sure to incubate for 10 minutes at room temperature after lysis. For proper lysis, the complete mix of sample and Buffer NVL is essential.
	Incorrect elution conditions Add nuclease-free water to the center of the spin column membrane and perform incubation for 1 minute before centrifugation.

	Precipitation of Buffer NVL Storage at low temperature may cause precipitation in Buffer NVL. For good results, any precipitate in the buffer should be dissolved completely by incubating the buffer at 37°C (or above) until it disappears.
	Degradation of RNA RNase can be introduced during use. Be certain not to introduce any RNases during the procedure or later handling. Keep tubes closed whenever possible during the preparation.
	Carrier RNA not added Add Carrier RNA at lysis step. Omission of Carrier RNA leads to low purification efficiency.
	Degradation of Carrier RNA Carrier RNA was not stored at -20°C or was affected by multiple freeze-thaw cycles. After reconstitution, Carrier RNA should be stored at -20°C.
	Buffer RBW and RNW used in the wrong order Ensure that Buffer RBW and RNW are used in the correct order in the protocol. If used in the wrong order, perform the last washing step with RNW.
Eluate does not perform will in downstream application	**Residual ethanol remains in the eluate** To remove any residual ethanol included in the Buffer RNW from spin column membrane, centrifuge again (see step 12).
	Buffer RBW and RNW used in the wrong order

	Ensure that Buffer RBW and RNW are used in the correct order in the protocol. If used in the wrong order, perform the last washing step with RNW.

Ordering Information

Product Name	Cat. No.	# of Preps
ABIOpure™ Total DNA Blood/Tissue/Cell	M501DP100	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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