ABIOpure™ Viral (version 2.0)

DNA/RNA Extraction Handbook

Cat No: M561VT50

FOR RESEARCH USE ONLY

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Kit Components

Component	M561VT50	Storage Temp
No. of preps	50	
Buffer BL*	15 ml	RT (15~25°C)
Buffer RB1	22 ml	RT (15~25°C)
Buffer BW	30 ml	RT (15~25°C)
Buffer TW	50 ml	RT (15~25°C)
Nuclease-free water	15 ml	RT (15~25°C)
Proteinase K*	13 mg	RT (15~25°C)
PK Storage Buffer	1 ml	RT (15~25°C)
Carrier RNA*	370 μg	RT (15~25°C)
ABIOpure™ column type micro S (with collection tube)	50 ea	RT (15~25°C)
1.5 ml microcentrifuge tube	50 ea	RT (15~25°C)

***** Refer to Stability and Storage and Reagent Preparation sections

Precautions

Buffer BL, RB1, and BW 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.

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

All components of ABIOpure™ Viral DNA/RNA Extraction kit should be stored at room temperature (15~25°C).

After reconstitution of Proteinase K with PK Storage Buffer, it should be stored at 4°C for preservation of activity. It can be stored at 4°C for 1 year without significant decrease in activity. However, for prolonged preservation of activity, storing at -20°C is recommended.

Dissolved Carrier RNA should be immediately used for experiments or frozen in aliquots at -20°C.

Under cool ambient conditions, a precipitate may form in Buffer BL. In this case, heat the bottle above 37°C to dissolve the precipitate.

ABIOpure™ Viral DNA/RNA Extraction kit is guaranteed until the expiration date printed on the product label.

General Description

ABIOpure™ Viral DNA/RNA Extraction kit is designed for the purification of total nucleic acids from viral samples such as cell-free fluid, cell-culture supernatant, plasma, serum, swab, urine, and virus-infected samples. Purified nucleic acids can be used directly for PCR, qPCR, RT-PCR, or any downstream application without further manipulation.

ABIOpure™ Viral DNA/RNA Extraction kit utilizes advanced silica-binding technology to purify total nucleic acids sufficiently pure for many applications. Viral samples are lysed in optimized buffer containing detergent and lytic enzyme. Under optimized binding conditions, nucleic acids in the lysate bind to a silica membrane and impurities pass through a membrane into a collection tube. The membranes

are washed with a series of alcohol-containing buffer to remove any trace of proteins, cellular debris and salts. Finally pure nucleic acids are released into a clean collection tube with deionized water or low ionic strength buffer. The eluate should be treated with care because nucleic acids are very sensitive to contaminants, such as nucleases, often found on general labware and dust. To ensure nucleic acids stability, it is recommended to store the eluate at 4°C for immediate analysis or to freeze at -70°C for long-term storage.

Limitations

The ABIOpure™ Viral DNA/RNA Extraction kit is intended for research use only applications.

Quality Control

All components of the ABIOpure™ Viral DNA/RNA 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
Operation time: ~ 20 min
Maximum volume of starting samples: 200 µl/prep
Maximum loading volume: 750 µl
Minimum elution Volume: 20 µl
Applications: RT-PCR, Automated Fluorescent DNA Sequencing, PCR, Sequencing and other enzymatic reactions.

Samples

Up to 200 µl of cell-free fluid, cell-culture supernatant, plasma, serum, swab, urine, or virus-infected samples.

Starting material, such as plasma or serum, should be stored at -70°C in aliquots for long term storage. Repeated freezing and thawing of frozen plasma 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.

Reagent Preparation

Carrier RNA

This kit is provided with carrier RNA which can be added to the lysis step if required. Carrier RNA enhances binding of nucleic acid 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 it 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.

Proteinase K

This kit provides Proteinase K and PK Storage Buffer for dissolving Proteinase K. Reconstituted Proteinase K provides efficient viral lysis for most sample types.

To obtain a solution of 20 mg/ml, add 650 µl of PK Storage Buffer to the tube of lyophilized Proteinase K, and mix carefully to avoid foaming. After reconstitution of Proteinase K with PK Storage Buffer, it should be stored at 4°C for preservation of activity. It can be stored at 4°C for 1 year without significant decrease in activity. For prolonged preservation of activity, storing at -20°C is recommended.

Materials required but not provided

- Laminar flow hood
- Pipette set (10 µl, 100 µl and 1000 µl)
- Sterile nuclease-free pipette tips with aerosol barriers
- Microcentrifuge and vortex
- PBS (phosphate-buffered saline) for certain samples
- Suitable protection (ex. lab coat, disposable gloves, goggles, etc.)

Protocol

1. **Pipet 10 µl of Proteinase K solution into the bottom of a 1.5 ml microcentrifuge tube.**

2. **Transfer up to 200 µl of sample to the tube.**

   If the sample volume is less than 200 µl, adjust the volume to 200 µl with PBS.

3. **Add 200 µl of Buffer BL to the tube.**

   In case of large sample volume, increase the amount of Buffer BL and Carrier RNA proportionally.

4. **Add 7 µl of Carrier RNA to the tube and mix thoroughly by vortex for 10 seconds.**

   It is essential to mix the sample and Buffer BL thoroughly for a good result.

	5.	**Incubate the tube at 56°C for 10 minutes.** Spin down briefly to remove any drops from inside of the lid.
	6.	**Add 400 µl of Buffer RB1 to the sample and mix thoroughly by vortex 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.
	7.	Transfer the **mixture to the spin column** carefully (column type micro S, white)
	8.	**Centrifuge at ≥ 10,000 x g for 1 minute at room temperature.** Discard the pass-through and reinsert the spin column back into the same tube. If the sample volume exceeds 750 µl, repeat steps 7 ~ 8 with the remainder of the sample.
	9.	**Add 500 µl of Buffer BW to the spin column.**
	10.	**Centrifuge at ≥ 10,000 x g for 1 minute at room temperature.** Discard the pass-through and reinsert the spin column back into the same tube.
	11.	**Add 700 µl of Buffer TW to the spin column.**
	12.	**Centrifuge at ≥ 10,000 x g for 1 minute at room temperature.**

		Discard the pass-through and reinsert the spin column back into the same tube.
	13.	**Centrifuge at full speed for 1 minute at room temperature to remove residual wash buffer.** **Transfer the 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 TW.
	14.	**Add 20 ~ 50 µl of Nuclease-free water to the center of the membrane in the spin column.** **Let it stand for 1 minute.**
	15.	**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 sample	Use more sample. Concentrate the sample volume to 300 µl using a microconcentrator.
	Sample not homogenized completely	For proper lysis, the complete mix of sample and Buffer BL 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 BL	Storage at low temperature may cause precipitation in Buffer BL. 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 afflicted with multiple freeze-thaw cycles. After reconstitution, Carrier

		RNA should be stored in aliquots at -20°C.
	BW and TW Buffers used in the wrong order	Ensure that Buffer BW and TW are used in the correct order in the protocol. If used in the wrong order, perform the last washing step with TW.
Eluate does not perform well in downstream application	Residual ethanol remains in eluate	To remove any residual ethanol included in Buffer TW from spin column membrane, centrifuge again for complete removal of ethanol (step 13).
	Buffer BW and TW used in the wrong order	Ensure that Buffer BW and TW are used in the correct order in the protocol. If used in the wrong order, perform the last washing step with TW.

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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