Axen™ Viral NA Kit II

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Product description

Axen™ Viral NA Kit II provides easy and rapid method for simultaneous isolation of pure DNA and RNA from cell-free fluid, cell culture medium, plasma, or serum, swab, urine, or virus-infected samples. Viral nucleic acids can be obtained in just 20 minutes without any use of hazardous organic solvent, and it can be directly used in various downstream applications, such as PCR, RT-PCR, without any further manipulations.

Kit contents

Contents [a]	Size		**Storage conditions**
Cat.No.	**MG-P-016-50**	**MG-P-016-200**	
No. of preparation	50	200	
Spin column and Collecting tube	50 ea	200 ea	Room Temperature
Buffer BL	15 mL	50 mL	
Buffer VB (concentrate) [b,c]	5 mL	20 mL	
Buffer RW1 (concentrate) [b,c]	15 mL	60 mL	
Buffer RW2 (concentrate) [c]	8 mL	32 mL	
Nuclease-free Water	15 mL	30 mL	
Carrier RNA [d]	300 ug	1.1 mg	2~8°C [d]
Proteinase K solution (20 mg/mL) [e]	0.52 mL	1.05 mL x 2	4°C

a. All components of this kit except carrier RNA solution and proteinase K solution should be stored at room temperature (15~25°C). Long exposure to heat source can deteriorate the performance of kit significantly.

b. During shipment or storage under cold ambient condition, a precipitate can be formed in buffer VB (conc.) and RW1 (conc.). Heat the bottle at 20~40°C to dissolve completely in such a case.

c. Buffer VB, RW1 and RW2 are provided as concentrate. Ethanol must be added before first use as the indication on the bottle label.

d. Carrier RNA should be stored under 4°C upon arrival for avoidance of degradation. After the reconstitution of carrier RNA solution, it is ideal to store under -20°C with dividing into small aliquot. Repeated freezing and thawing will degrade the RNA.

e. Proteinase K solution should be stored under 4°C on arrival for conservation of activity. Proteinase K solution can be stored at 4°C for a year without significant decrease of performance, but it can be stored under -20°C for prolonged conservation of activity.

Product use limitations

Axen™ Viral NA Kit II is intended for research uses only. This kit is not intended for diagnosis or treatment for human. All due care and attention should be exercised in the handling of the products.

Safety information

Axen™ Viral NA Kit II contains irritants which are harmful when in contact with skin or eyes, or inhaled or swallowed. Care should be taken when handling this product. Always wear gloves and eye protection, and follow standard safety precautions.

Buffer BL, VB and RW1 contain chaotropes which can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solutions directly to the sample-preparation waste.

Preventing RNase Contamination

RNase can be introduced accidentally during RNA preparation. Various factors such as reagents, air, dust, and human hands or skin, can be the sources of RNase contamination. Always wear disposable gloves and use sterile, disposable plastic wares. Use the pipette reserved for RNA work. Maintain a separate area for RNA work. Carefully clean the surfaces.

Reconstitution of carrier RNA solution

Carrier RNA is provided in a lyophilized form. In order to reconstitute the carrier RNA solution to 1 ug/uL, add 1 uL of distilled water per 1 ug of carrier RNA. For instance, add 300 uL of distilled water into 300 ug of carrier RNA.

The reconstituted carrier RNA solution should be stored at low temperature for avoidance of degradation. It is ideal to store under -20°C with dividing into small aliquot. Note that repeated freezing and thawing will degrade the RNA.

Preparation of ethanol-added buffer

Buffer VB, RW1 and RW2 are provided as concentrate. Absolute ethanol (ACS grade or better) should be added as below before first use.

Buffer name	Included in	Volume of Contents	**Ethanol to be added**	The final volume
VB	MG-P-016-50	5 mL	**20 mL**	25 mL
	MG-P-016-200	20 mL	**80 mL**	100 mL
RW1	MG-P-016-50	15 mL	**15 mL**	30 mL
	MG-P-016-200	60 mL	**60 mL**	120 mL
RW2	MG-P-016-50	8 mL	**32 mL**	40 mL
	MG-P-016-200	32 mL	**128 mL**	160 mL

Procedure for purification of DNA/RNA from viral sample

To check before start

- Prepare the water bath or dry bath to 56°C
- Required consumables; 1.5 mL centrifuge tube

- Do NOT use the precipitated buffers. If a precipitate forms in a buffer, dissolve completely at 20~40°C before use.

!!! Before first use, add the indicated volume of absolute ethanol to buffer VB, RW1, and RW2 as below.

1. **Place 200 uL of the sample and 10 uL of proteinase K solution into 1.5 mL microcentrifuge tube.**
   - *A sample can be used as forms of swab-storage media, cell-free fluid, cell culture media, plasma, serum, and urine.*
   - *Adjust the sample volume to 200 uL with 1x PBS, if the volume is less than 200 uL.*
2. **Add 200 uL of buffer BL and 5 uL of carrier RNA solution to the tube and mix thoroughly for 15 secs by vortexing.**
   - *It is essential for proper lysis to make the mixture homogenized.*
3. **Incubate the lysate for 10 mins at 56˚C.**
   - *After incubation, briefly centrifuge the tube to remove drops from the inside of the lid.*
4. **Add 400 uL of buffer VB to the lysate and mix thoroughly for 10 secs by vortexing.**
   - *Do NOT centrifuge after addition of buffer VB.*
5. **Transfer the mixture into a spin column, centrifuge for 30 secs at 13,000 xg, discard the pass-through, and insert the column back into the collection tube.**

6. **Apply 500 uL of buffer RW1 into the column and centrifuge for 30 secs at 13,000 xg, discard the pass-through, and insert the column back into the collection tube.**
7. **Apply 700 uL of buffer RW2 into the column and centrifuge for 30 secs at 13,000 xg, discard the pass-through, and insert the column back into the collection tube.**
8. **Centrifuge for 1 min at 13,000xg (or full speed) for drying the membrane and transfer the spin column into a new 1.5 mL tube.**
   - *Residual wash buffer may interfere with downstream applications. Care must be taken not to be contaminated by the carryover of buffer RW2.*
9. **Apply 30 ~ 50 uL of Nuclease-free water to the center of spin column membrane and let it stand for 1 min.**
   - *Elution volume can be adjusted according to the purpose of experiment.*
   - *Note that at least 30 uL of eluent must be applied because the lesser eluent will not soak the membrane entirely, followed by poor or unpredictable result.*
10. **Centrifuge at 13,000 xg for 1 min for eluting.**
    - *Purified nucleic acid can be stored at 4°C for immediate analysis or it can be stored at -70°C for long term storage.*

Trouble shooting guide

Facts	*Possible causes*	Recommendations
Low yield	*Low viral titer in the sample*	Use more sample. Concentrate the larger sample to 200 uL using micro-concentrator.
	Poor quality of starting material	Use a freshly harvested sample or well-conserved sample if possible. Repeated freezing and thawing of sample should be avoided.
	Sample not homogenized	For proper lysis, it is essential to get homogenate by mixing completely with buffer BL.
	Ethanol was not added to the buffers	Buffers VB, RW1 and RW2 are provided as concentrate. Ethanol must be added to these buffers before first use. If not, the result will be significantly poor.
	Incorrect pipetting of eluent	Make sure to pipet Nuclease-free water onto the center of the spin column membrane. It is important to soak the entire membrane up the eluent.
Enzymatic reaction is not performed well with purified RNA	*Salt carryover in eluate*	Ensure that washing steps are carried out just in accordance with the protocols. Additional RW2-washing step may help remove salts from the membrane.
	Residual ethanol in eluate	The spin column membrane should be dried completely before eluting. Perform additional centrifugation to dry the membrane, if needed. Do NOT incubate the column at high temperature.


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