Axen™ Viral NA Kit

Product description

Axen™ Viral NA kit provides easy and rapid method for simultaneous isolation of pure RNA and DNA from cell-free fluid, cell culture medium, plasma, or serum, swab, urine, or virus-infected liquid samples. Pure nucleic acids can be obtained in just 15 minutes without any use of hazardous organic solvent. Purified RNA and DNA can be directly used in various downstream applications, such as PCR, RT-PCR, without any further manipulations.

Kit contents

Contents*	Size		**Storage conditions**
Cat. No.	**MG-P-015-50**	**MG-P-015-200**	
No. of preparation	50	200	
Mini column and Collecting tube†	50 ea	200 ea	Room Temperature
Buffer VL**	30 mL	110 mL	
Buffer VB(concentrate)**,***	8 mL	30 mL	
Buffer RW1 (concentrate)**,***	15 mL	55 mL	
Buffer RW2 (concentrate)***	8 mL	32 mL	
Nuclease-free Water	15 mL	30 mL	

* All components of this kit should be stored at room temperature (15~25°C). Long exposure to heat source can deteriorate the performance of kit significantly.

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

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

† Although the spin column can be stored at room temperature, it is ideal to store under cool ambient condition or in a refrigerator. Heat sources and direct sunlight must be avoided.

Product use limitations

Axen™ Viral NA kit 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 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 VL, 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.

Procedure for purification of DNA/RNA from viral sample

To check before start

- Required consumables; 1.5 mL or 2 mL micro centrifuge tube
  Absolute ethanol, ACS grade or better

- 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.

Buffer name	Included in	Volume of Contents	**Ethanol to be added**	The final volume
VB	MG-P-015-50	8 mL	**32 mL**	40 mL
	MG-P-015-200	30 mL	**120 mL**	150 mL
RW1	MG-P-015-50	15 mL	**15 mL**	30 mL
	MG-P-015-200	55 mL	**55 mL**	110 mL
RW2	MG-P-015-50	8 mL	**32 mL**	40 mL
	MG-P-015-200	32 mL	**128 mL**	160 mL

1. **Transfer up to 300 uL of sample into a 1.5 mL or 2 mL micro tube.**
   - *A sample can be used as forms of swab-storage media, cell-free fluid, cell culture media, plasma, serum, urine, or other body fluid.*
   - *When the sample is less than 300 uL, adjust the volume to 300 uL with 1x PBS or reduce the volume of the buffer VL and VB proportionally.*
2. **Add 500 uL of buffer VL to the tube and lyse the sample by pipetting or vortexing**
   - *It is critical for proper lysis to make the mixture homogenized.*
3. **Incubate the lysate for 10 mins at room temperature.**
   - *After incubation, briefly centrifuge the tube to remove drops from the inside of the lid.*
4. **Add 700 uL of buffer VB to the lysate and mix thoroughly by vortexing or inverting.**
   - *Do NOT centrifuge after addition of buffer VB*

5. **Transfer up to 750 uL of 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. **If there is a remaining mixture, repeat the step 5 with them.**
7. **Apply 500 uL of buffer RW1 into the column and centrifuge for 30 secs at 13,000 xg.**
8. **Remove the spin column, discard the pass-through, and insert the column back into the collection tube.**
9. **Apply 700 uL of buffer RW2 into the column and centrifuge for 30 secs at 13,000 xg.**
10. **Remove the spin column, discard the pass-through, and insert the column back into the collection tube.**
11. **Centrifuge for 1 min at 13,000xg 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.*
12. **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.*
13. **Centrifuge at 13,000 xg for 1 min for eluting.**
    - *Purified nucleic acid can be stored at 4°C for immediate analysis and 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 300 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 VL.
	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 to the center of the spin column membrane.
Spin column clogged	*Cryoprecipitate in the sample*	Some thawed plasma sample can have cryoprecipitate in it, and it can clog the pore of the membrane during experiment. Cryoprecipitate can be removed from the sample by centrifugation.
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.