Axen™ Viral NA Kit III

Axen™ Viral NA Kit III

Product description

Axen™ Viral NA Kit III provides simple and fast method for simultaneous isolation of pure RNA and DNA from cell-free fluid, cell culture medium, plasma, or serum, swab, urine, or virus-infected 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-017-50**	**MG-P-017-200**	
No. of preparation	50	200	
Spin column with collecting tube**	50 ea	200 ea	2~8˚C**
Buffer VN***	20 mL	75 mL	Room Temperature
Buffer VB(concentrate)***,****	5 mL	16 mL	
Buffer RW1 (concentrate)***,****	15 mL	60 mL	
Buffer RW2 (concentrate)****	8 mL	32 mL	
Nuclease-free Water	5 mL	30 mL	
Carrier RNA*****	300 ug	1.1 mg	2 ~ 8˚C

* All components should be stored at the proper condition as described. Basically, long exposure to heat source can deteriorate the performance of kit significantly.

** Spin column can be stored under cool ambient condition. Avoid heat sources and direct sunlight. Ideally, it should be stored in a refrigerator shielded from light.

*** During delivery or storage under cold ambient condition, a precipitate can be formed in buffer VN, VB (conc.) and/or RW1 (conc.). Before use, heat the bottle at 20~40˚C to dissolve completely.

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

***** 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.

Product use limitations

Axen™ Viral NA Kit III 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 III 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 VN, 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 before first use as below.

Buffer name	Included in	Volume of Contents	**Ethanol to be added**	The final volume
VB	MG-P-017-50	5 mL	**20 mL**	25 mL
	MG-P-017-200	16 mL	**64 mL**	80 mL
RW1	MG-P-017-50	15 mL	**15 mL**	30 mL
	MG-P-017-200	60 mL	**60 mL**	120 mL
RW2	MG-P-017-50	8 mL	**32 mL**	40 mL
	MG-P-017-200	32 mL	**128 mL**	160 mL

Procedure for purification of DNA/RNA from viral sample

To check before start

- 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 described on previous page.

1. **Place 300 uL of buffer VN and 5 uL of carrier RNA solution into a 1.5 mL microcentrifuge tube.**
2. **Apply up to 100 uL of sample into the tube.**
   - *A sample can be applied as forms of swab-storage media, cell-free fluid, cell culture media, plasma, serum, and urine.*
   - *If the sample volume is less than 100 uL, adjust the volume to 100 uL with 1x PBS.*
3. **Mix thoroughly by vortexing for 10 secs.**
   - *It is essential for proper lysis to make the mixture homogenized.*
4. **Incubate the lysate for 10 mins at room temperature.**
   - *After incubation, briefly centrifuge the tube to remove drops from the inside of the lid.*
5. **Add 350 uL of buffer VB into the lysate and mix thoroughly by vortexing for 10 secs.**
   - *Do NOT centrifuge after addition of buffer VB.*
6. **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.**
   - *If the mixture has remained, repeat the step 6 with them.*

7. **Apply 500 uL of buffer RW1 into the column and centrifuge for 30secs 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 30secs 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 (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.*
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 (or full speed) 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 volume or concentrate the larger sample to 100 uL using a micro-concentrator.
	Poor quality of starting material	Use a freshly collected 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 VN.
	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.


Humanizing Genomics
macrogen