

AxyPrep Body Fluid Viral DNA/RNA Miniprep Kit

Kit contents, storage and stability

Cat. No.	AP-MN-BF-VNA-50	AP-MN-BF-VNA-250
Kit Size	50 preps	250 preps
AxyPrep column	50	250
2 ml microfuge tube	50	250
1.5 ml microfuge tube	50	250
Buffer V-A	30 ml	150 ml
Buffer V-B	7 ml	35 ml
Buffer AP2	7 ml	35 ml
Buffer W1A concentrate	24 ml (+17 ml ethanol)	120 ml (+85 ml ethanol)
Buffer W2 concentrate	24 ml	2 × 72 ml
Buffer TE (DNase/RNase-free)	4 ml	20 ml
Protocol Manual	1	1

All buffers are completely stable for a period of at least 12 months from the date of receipt when stored under ambient conditions. Please avoid exposure to direct sunlight or extremes in temperature.

Buffer V-A: Virus lysis buffer. Store at room temperature.

Buffer AP2: Protein-deleting buffer. Store at room temperature.

Buffer V-B: Protein precipitation buffer. Store at room temperature.

Buffer W1A concentrate: Wash buffer. Store at room temperature. Before use, add the amount of ethanol specified on the bottle label to the W1A concentrate. Either 100% or 95% (denatured) ethanol can be used.

Buffer W2 concentrate: Desalting buffer. Before use, add the amount of ethanol specified on the bottle label to the W2 concentrate. Either 100% or 95% (denatured) ethanol can be used.

Buffer TE: 5 mM Tris-HCl, pH8.5, 0.1 mM EDTA, DNase- and RNase-free. Store at room temperature.

Introduction

Viruses not only can cause serious health troubles but also play an important role in molecular biology and biomedical research. Efficient isolation of viral DNA and viral RNA with high purity and integrity is often a challenge. The AxyPrep Viral DNA/RNA Miniprep Kit provides a rapid method for

the purification of viral nucleic acid from 250 µl of body fluid, including plasma, serum, ascites, cell culture supernatant, cerebrospinal fluid, urine, etc.

Buffer V-A efficiently lyses the viral particles existing in body fluids. During lysis, viral DNA and viral RNA are released and the infective nature of the virus is eliminated. Proteins and PCR inhibitors are removed by precipitation with Buffer V-B. Viral nucleic acid remains soluble in the supernatant and is purified by binding to a special nonorganic membrane in the AxyPrep column. After brief washes with Buffer W1A and Buffer W2 to remove residual impurities and salt, the purified viral nucleic acid is then eluted in Buffer TE and can be used immediately. The nucleic acid purified by this method is free from contaminants, such as proteins, pigments, lipids and PCR/RT-PCR inhibitors, and it is especially suitable for demanding PCR/RT-PCR analyses.

Caution

1. Before proceeding with this procedure, make all required preparations to avoid infection by body fluid-borne viral agents. Please follow local guidelines for working with body fluids and infectious agents.
2. Strictly follow all steps in the protocol, and put all waste in an appropriate Biohazardous Waste container Autoclave.
3. Buffer V-A and Buffer W1A contain chemical irritants. When working with the buffers, always wear suitable protective clothing such as safety glasses, laboratory coat and gloves. Be careful to avoid contact with eyes and skin. In the case of such contact, wash immediately with water and seek medical assistance if necessary.

Equipment and consumables required

- Microcentrifuge capable of 12,000 × g
- 100% or 95% (denatured) ethanol

Preparation before experiment

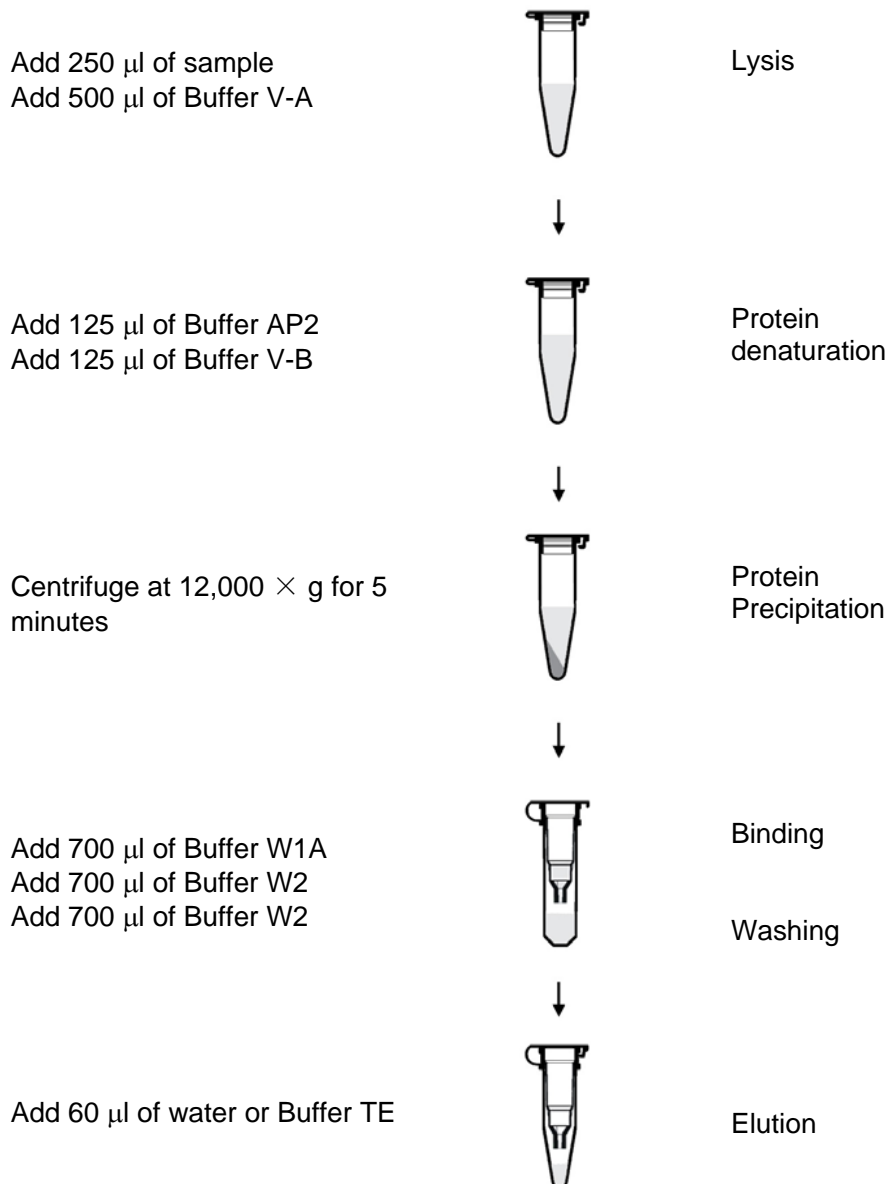
Before using the kit, add the amount of ethanol specified on the bottle label to the Buffer W1A and Buffer W2 concentrate and mix well. Either 100% or 95% (denatured) ethanol can be used.

Protocols

This protocol is designed for the preparation of viral DNA or RNA from 250 µl of body fluid. For preparation of viral nucleic acid from other body fluid volumes, Buffer V-A, Buffer V-B and Buffer AP2 should be added in proportion. However, the Buffer W1A and Buffer W2 volumes will remain unchanged. Contamination with nucleic acid will cause false-positive in PCR/RT-PCR. All plasticware, reagents and gloves used in the experiments should be free from contamination with DNA and RNA. If this kit is used for the preparation of viral RNA, use RNase-free pipette tips and 1.5 ml microfuge tubes.

1. Collect 250 μ l of a body fluid sample in a 1.5 ml microfuge tube (not provided).
2. Add 500 μ l of Buffer V-A. Vortex to mix well and incubate at room temperature for 5 minutes.
3. Add 125 μ l of Buffer AP2.
4. Add 125 μ l of Buffer V-B and mix vigorously. Centrifuge at 12,000 \times g for 5 minutes.
Note: Buffer V-B is viscous. Pipette slowly to aspirate the required volume.
5. Place an AxyPrep column into a 2 ml microfuge tube (provided). Transfer 850 μ l of the clarified supernatant from Step 4 into the AxyPrep column. Centrifuge at 5,000 \times g for 1 minute.
Note: Extend centrifuge time or increase centrifuge force if lysate is remained in the AxyPrep column.
6. Discard the filtrate in the 2 ml microfuge tube. Place the AxyPrep column back into the 2 ml microfuge tube. Add 700 μ l of Buffer W1A to the AxyPrep column, stand at room temperature for 1 minute and centrifuge at 6,000 \times g for 1 minute.
Note: Make sure that 95-100% ethanol has been added into Buffer W1A concentrate. Make a notation on the bottle label for future reference.
Note: Extend centrifuge time or increase centrifuge force if lysate is remained in the AxyPrep column.
7. Discard the filtrate and place the AxyPrep column back into the 2 ml microfuge tube. Add 700 μ l of Buffer W2 and centrifuge at 12,000 \times g for 1 minute.
Note: Make sure that 95-100% ethanol has been added into Buffer W2 concentrate. Make a notation on the bottle label for future reference.
8. **Optional Step:** Discard the filtrate from the 2 ml microfuge tube. Place the AxyPrep column back into the 2 ml microfuge tube. Add 700 μ l of Buffer W2 to the AxyPrep column and centrifuge at 12,000 \times g for 1 minute.
Note: Two washes with Buffer W2 are used to ensure the complete removal of salt, eliminating potential problems in subsequent enzymatic reactions.
9. Discard filtrate from 2 ml microfuge tube. Place the AxyPrep column back into the 2 ml microfuge tube. Centrifuge at 12,000 \times g for 1 minute.
10. Transfer the AxyPrep column in another clean 1.5 ml microfuge tube (provided). To elute the viral DNA/RNA, add 60 μ l of Buffer TE to the center of the membrane and let it stand for 1 minute at room temperature. Centrifuge at 12,000 \times g for 1 minute.
Note: To prevent viral RNA degradation, it is recommended to add RNasin at 1 unit/ μ l in TE eluent when viral RNA is purified.

Overview



Troubleshooting

1. PCR or RT-PCR generates multiple amplicons or wrong size amplicon

This problem is generally attributable to contamination of the plasticware or PCR reagents. If amplicons are present in addition to the correct amplicon, there is most likely a contamination issue. However, a negative controls (absence of viral DNA/RNA template) should be run to verify the source of the contamination. Plasticware and/or reagents should be replaced as necessary.

2. PCR fails to generate amplicons

Problem with PCR primers, reagents or cycling parameters

Run the appropriate control reactions to verify the integrity of all PCR components.

Failure to add ethanol to W2 concentrate (premature elution of bound viral DNA)

Inspect the volume of W2 in the bottle or review any notes made on the label to confirm the addition of ethanol. Inspect ethanol source to confirm that it was 95% (denatured) or 100% ethanol...NOT 70% ethanol. If the Buffer W2 is suspect, the procedure can be repeated, substituting 70% ethanol for the W2.

Low viral titer in sample

Increase the amount of viral DNA to the maximum extent possible and repeat the PCR. Increase the number of cycles in the PCR. Set up a control PCR in which the viral DNA is replaced with another template

Degradation of viral DNA

The limited amounts of viral nucleic acid present in most biological fluid samples demands that care and cleanliness be exercised when purifying. If necessary, repeat the procedure after carrying out the appropriate measures to remove any potential sources of contamination, etc.

3. RT-PCR fails to generate amplicons

Problem with RT-PCR primers, reagents or cycling parameter

Run the appropriate control reactions to verify the integrity of all RT-PCR components.

Failure to add ethanol to W2 concentrate (premature elution of bound viral RNA)

Inspect the volume of W2 in the bottle or review any notes made on the label to confirm the addition of ethanol. Inspect ethanol source to confirm that it was 95% (denatured) or 100% ethanol...NOT 70% ethanol. If the W2 Buffer is suspect, the procedure can be repeated, substituting 70% ethanol for the W2.

Low viral titer in sample

Increase the amount of viral RNA to the maximum extent possible and repeat the RT-PCR. Increase the number of cycles in the RT-PCR. Set up a control RT-PCR in which the viral DNA is replaced with another template.

Degradation of viral RNA

Given the limited amount of viral RNA which is likely to be recovered from biologic fluid samples, it is particularly important that great care and cleanliness be exercised when preparing, storing and handling these samples. If necessary, repeat the procedure after carrying out the appropriate measures to remove any potential sources of contamination, etc. Use DEPC-treated materials whenever practical. Add RNasin (1 unit/ μ l) to the purified viral RNA for stability during storage.