

AxyPrep Body Fluid Viral DNA/RNA Miniprep Kit

Kit contents, storage and stability

Cat. No.	AP-MN-BF-VNA-4	AP-MN-BF-VNA-50	AP-MN-BF-VNA-250
Kit size	4 preps	50 preps	250 preps
Miniprep column	4	50	250
2 ml Microfuge tube	8	100	500
1.5 ml Microfuge tube	4	50	250
Poly (A)	6 µg	80 µg	400 µg
2-Mercaptoethanol	120 µl	120 µl	120 µl
Buffer V-L	1 ml	12 ml	60 ml
Buffer V-N	1 ml	4 ml	20 ml
Buffer W1A concentrate	2.4 ml	24 ml	120 ml
Buffer W2 concentrate	2.4 ml	24 ml	72 ml
Buffer TE (DNase & RNase-free)	1 ml	4 ml	20 ml
Protocol manual	1	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. Axygen Biosciences warrants the performance of this product for a period of 12 months from the date of receipt when stored under the specified conditions.

Poly (A) : Polyadenylic acid. Store at -20°C.

Buffer V-L: Viral lysis buffer. Store at 4°C.

Buffer V-N: Protein-removal buffer. Store at room temperature.

Buffer W1A concentrate: Wash buffer. 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. Store at room temperature.

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 (DNase & RNase-free): 5 mM Tris-HCl, pH 8.5, 0.1 mM EDTA, DNase- and RNase-free. Store at room temperature.

2-Mercaptoethanol: Store at room temperature.

Introduction

Viruses are not only potentially pathogenic, 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 simple, rapid and efficient

method for the purification of viral nucleic acid from 200 μ l of body fluid, including plasma, serum, ascites, cell culture supernatant, cerebrospinal fluid, urine, etc.

Buffer V-L efficiently lyses the viral particles present 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-N. Viral nucleic acid remains soluble in the supernatant and is purified by binding to a special Miniprep 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 (DNase & RNase-free) and can be used immediately. The nucleic acid purified by this method is free from contaminants, such as proteins, pigments, lipids and quantitative PCR/RT-PCR inhibitors, and it is especially suitable for demanding PCR/RT-PCR analyses. Both viral DNA and RNA are simultaneously purified by this method.

Precautions

- 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 and autoclave.
- 3) Buffer V-L, Buffer V-N 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 \times g
- 100% or 95% (denatured) ethanol
- Glacial acetic acid
- Isopropanol
- 2-Mercaptoethanol

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.
- Before using the kit, add the Poly(A) to Buffer V-L and add 2-mercaptoethanol into Buffer V-L to a final concentration at 0.1%. Mix well and store at 4°C.
- Prepare [isopropanol+1% acetic acid] by combining 99 ml of isopropanol (95-100%) + 1 ml of glacial acetic acid.

Protocols

This protocol is designed for the preparation of viral DNA or RNA from 200 μ l of body fluid. For preparation of viral nucleic acid from other body fluid volumes, Buffer V-L and Buffer V-N should be

added in proportion. However, the Buffer W1A and Buffer W2 volumes will remain unchanged. Contamination with nucleic acid may cause false-positives in the 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 microfuge tubes.

1. Collect 200 μ l of a body fluid sample in a 1.5 ml microfuge tube.
Note: Nucleic acid present in any contaminating bacteria or cells present in the body fluid sample will be copurified with the viral DNA/RNA. While this generally does not interfere with the PCR or RT-PCR amplification and results, it may be desirable to remove bacteria or cells by subjecting the sample to a 5 minute high-speed centrifugation at 12,000 \times g, before proceeding with the purification. Following centrifugation, carefully transfer the supernatant to a clean tube without disturbing the bottom of the first tube or any discernable pellet.
2. Add 200 μ l of Buffer V-L. Mix vigorously and thoroughly. Incubate at room temperature for 5 minutes.
Note: Make sure that Ploy (A) and 2-mercaptoethanol have been added into Buffer V-L.
3. Add 75 μ l of Buffer V-N, vortex to mix well. Centrifuge at 12,000 \times g for 5 minutes.
4. Transfer the clarified supernatant from Step 3 into a 2 ml microfuge tube (provided), and add 250 μ l [Isopropanol+1% acetic acid] and mix well.
5. Place a Miniprep column into a 2 ml microfuge tube (provided). Transfer the clarified liquid from Step 4 into the Miniprep column. Centrifuge at 6,000 \times g for 1 minute.
Note: Increase the centrifuge time or g-force if lysate remains in the Miniprep column after 1 minute.
6. Discard the filtrate in the 2 ml microfuge tube. Place the Miniprep column back into the 2 ml microfuge tube. Add 500 μ l of Buffer W1A to the Miniprep column and let it stand at room temperature for 1 minute. Centrifuge at 12,000 \times g for 1 minute.
Note: Increase the centrifuge time or g-force if lysate remains in the Miniprep column after 1 minute.
7. Discard the filtrate and place the Miniprep column back into the 2 ml microfuge tube. Add 800 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. Discard the filtrate from the 2 ml microfuge tube. Place the Miniprep column back into the 2 ml microfuge tube. Centrifuge at 12,000 \times g for 1 minute to remove residual Wash Buffer.
9. Transfer the Miniprep column to a clean 1.5 ml microfuge tube (provided). To elute the viral DNA/RNA, add 40-60 μ l of Buffer TE (DNase & RNase-free) to the center of the membrane and let it remain for 1 minute at room temperature. Centrifuge at 12,000 \times g for 1 minute.

Overview

Optional: 12,000×g for 5 minutes

Add 200 µl of sample
Add 200 µl of Buffer V-L



Lysis



Add 75 µl of Buffer V-N



Protein
denaturation



Centrifuge at 12,000×g for 5 minutes

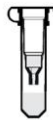
Add 250 µl [Isopropanal + 1% acetic
acid]



Protein
precipitation



Add 500 µl of Buffer W1A
Add 800 µl of Buffer W2



Binding
Washing



Add 40-60 µl of Buffer TE (DNase &
RNase-free)



Elution

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 control (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.

Alternatively, the body fluid sample may have contained contaminating bacteria or human cells whose nucleic acid was copurified with the viral DNA/RNA. To avoid any potential problem, repeat the preparation but centrifuge the body fluid sample as indicated in Step 1 of the protocol before proceeding.

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 Buffer W2 concentrate (premature elution of bound viral DNA)

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

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.

Warranty/Disclaimer

Axygen Biosciences warrants that this kit will perform as indicated for the specified application for a period of up to 12 months from the date of receipt when stored in the specified manner and used according to the instructions provided. In using this product, the customer agrees that Axygen Biosciences shall not be held liable for any direct or indirect damages, including, but not limited to, personal injury, property damage or lost profits (or other economic loss) resulting from the use or inability to use this product. In the event that this product fails to perform in the specified manner, remedial measures on the part of Axygen Biosciences shall be limited to the replacement of this product and will be implemented at the discretion of Axygen Biosciences.