UE Body Fluid Viral DNA/RNA Miniprep Kit

The kit provides a simple, rapid and efficient method for the purification of viral nucleic acid from 200 µl of body fluid, including plasma, serum, ascites, cell culture supernatant, cerebrospinal fluid, urine, etc. 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.

1. Kit contents, storage and stability

Cat. No.	UE-MN-BF-VNA-10	UE-MN-BF-VNA-50	UE-MN-BF-VNA-250
Kit size	**10 preps**	**50 preps**	**250 preps**
Miniprep column	**10**	**50**	**250**
2 ml microfuge tube	**20**	**100**	**500**
1.5 ml microfuge tube	**10**	**50**	**250**
Buffer V-L	**3 ml**	**12 ml**	**60 ml**
Buffer V-N	**1 ml**	**4 ml**	**20 ml**
Buffer W1A concentrate	**5 ml**	**24 ml**	**120 ml**
Buffer W2 concentrate	**5 ml**	**24 ml**	**72 ml**
Buffer TE (nuclease-free)	**1 ml**	**4 ml**	**20 ml**
Protocol manual	**1**	**1**	**1**

Buffer V-L: Viral lysis buffer. Store at room temperature.

Buffer V-N: Protein precipitation 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 (nuclease-free): 5 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. Store at room temperature.

2. Caution

1. Before proceeding with this procedure, make all required preparations to avoid infection by bodyfluid-borne viral agents. Please follow local guidelines for working with body fluids and infectiousagents.
2. Strictly follow all steps in the protocol, and put all waste in an appropriate Biohazardous Wastecontainer and autoclave.

3. In order to avoid false positives due to contamination of other nucleic acids, DNA and RNA-free centrifuge tubes, pipettes, tips, reagents and gloves must be used, and staff wear masks for operation.
4. Buffer V-L, Buffer V-N and Buffer W1A contain chemical irritants. When working with these 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.

3. Preparation before experiment

1) Before using the kit, add the amount of ethanol specified on the bottle label to the Buffer W1A and Buffer W2 concentrates and mix well.
2) Prepare [isopropanol+1% acetic acid] by combining 99 ml of isopropanol + 1 ml of glacial acetic acid.
3) If purifying viral RNA, please use RNase free tips and 1.5ml tube, or treat tips and 1.5ml tube with 0.1% DEPC water before use to avoid degradation of viral RNA by RNAse during purification. When purifying viral RNA, it is recommended to add RNasin to the eluent.

4. Protocols

This protocol is designed for the preparation of viral DNA or RNA from 200 µl 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, the buffer usage in subsequent steps remains unchanged.

1. Collect 200 µl 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 12,000xg, 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 µl of Buffer V-L. Mix vigorously and thoroughly. Incubate at room temperature for 5 minutes.
3. Add 75 µl of Buffer V-N, vortex to mix well. Centrifuge at 12,000xg for 5 minutes.
4. Transfer the clarified supernatant from Step 3 into a 2 ml microfuge tube (provided), and add 300µl [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 6,000xg 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 µl of Buffer W1A to the Miniprep column and let it stand at room temperature for 1 minute. Centrifuge at 12,000xg for 1 minute.

Note: Make sure that ethanol has been added to the W1A concentrate.

7. Discard the filtrate and place the Miniprep column back into the 2 ml microfuge tube. Add 800 µl of Buffer W2 and centrifuge at 12,000xg for 1 minute.

Note: Make sure that ethanol has been added into Buffer W2 concentrate.

8. Discard the filtrate from the 2 ml microfuge tube. Place the Miniprep column back into the 2 ml microfuge tube. Centrifuge at 12,000xg 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 µl of Buffer TE (nuclease-free) to the center of the membrane and let it remain for 1 minute at room temperature. Centrifuge at 12,000xg for 1 minute.

Note: When purifying viral RNA, it is recommended to add RNasin to the eluent at a concentration of 1 unit/ul.

5. Overview