艾科瑞生物
Accurate Biology

Instruction for Use

SteadyPure Virus DNA/RNA Extraction Kit

AG 21021
Version.V1E1

Research Use Only
Not For Diagnosis Procedures

1. Description

This kit utilizes high-binding centrifuge adsorption columns and a unique viral lysis system, making it suitable for extracting viral (Virus) DNA/RNA from whole blood, plasma, serum, cell culture supernatants, viral stocks, infected tissues, and other cell-free body fluids. The kit features high yield, simple operation, and rapid processing. It also includes Carrier RNA to protect trace amounts of viral DNA/RNA from degradation. The viral DNA/RNA extracted using this kit is of high purity and stable quality, making it suitable for various molecular biology experiments, including restriction enzyme digestion, PCR, library construction, Southern blotting, and microarray analysis.

2. Kit Information

Kit Name	Cat. No	Specification
SteadyPure Virus DNA/RNA Extraction Kit	AG 21021	50 rxns

3. Transportation and Storage

	Package 2-1	Package 2-2
Storage	Store at -20°C	Store at Room Temperature
Transportation	Transport at -20°C	Transport at Room Temperature

4. Kit Components

Package 2-1 (-20 °C Storage)

Kit Components	Volume
Proteinase K (20 mg/ml)	1 ml
Carrier RNA (6 μg/μl)	50 μL

Package 2-2 (Room Temperature Storage)

Kit Components	
Buffer VLS	13 ml
Buffer RWA	25 ml
Buffer RWB*1	27 ml
RNase Free Water*2	13 ml
Virus DNA/RNA Mini Columns	50 sets
Collection Tubes	50 pcs
RNase Free Tubes*3	50 pcs

*1: Before first use, add 63 ml of 100% ethanol to Buffer RWB (the volume ratio of Buffer RWB to absolute ethanol is 3:7), mix thoroughly, and label the bottle accordingly. Store at room temperature.

*2: It is recommended to store this component at -20°C after opening.

*3: This component is for eluting DNA/RNA only. Centrifuge tubes for the initial lysis must be provided by the user.

5. Not Provided Experimental Materials

Required materials: Absolute ethanol, sterile water (or PBS or 0.9% NaCl solution), 1.5 ml centrifuge tubes (RNase-free), water bath.

Note: Before first use, add 63 ml of 100% ethanol to Buffer RWB (the volume ratio of Buffer RWB to absolute ethanol is 3:7), mix thoroughly, and label the bottle accordingly. Store at room temperature.

6. Recommendation and Experimental Precautions

(1) Fresh experimental materials should be used whenever possible to ensure that the extracted DNA/RNA is not degraded.

(2) When grinding tissue materials with liquid nitrogen, liquid nitrogen should be continuously added to ensure that the extracted DNA/RNA is not degraded.

(3) The sample amount should not exceed the maximum starting amount, as this may clog the Mini Column and affect the yield and purity of the DNA/RNA.

(4) When extracting samples containing cells, such as whole blood and tissues, some cellular DNA/RNA may be released during lysis. Homology should be avoided when designing primers for subsequent detection.

(5) The adsorption column should be removed vertically during the experiment to prevent the column tip from touching the walls of the collection tube.

(6) Usage of Carrier RNA:

1. Carrier RNA can protect trace nucleic acids from degradation, thereby increasing the yield of viral DNA/RNA. Follow the ratio described in step 2 of the "Viral Lysis" section for addition. If the lysis system is scaled up, the amount of Carrier RNA should be increased proportionally.
2. Carrier RNA is derived from E. coli RNA. Homology should be avoided when designing primers for subsequent detection. If the target gene is homologous to Carrier RNA, false positives may occur (criteria for false positives: no sample is added during extraction, sterile water is used instead, and clear amplification is observed after PCR).
3. If false positives occur with Carrier RNA, primers can be redesigned, or Carrier RNA can be omitted. However, omitting Carrier RNA may result in a lower yield of trace viral DNA/RNA.
4. Detection of viral DNA/RNA can be performed using PCR, RT-PCR, or qPCR.

7. Protocol

7.1 Lysis [Different experimental materials require different lysis steps, as detailed below]

Plasma, Serum, Saliva, Viral Stocks, or Other Cell-free Body Fluids:

1. Transfer 10-200 µL of plasma, serum, saliva, viral stock, or other cell-free body fluid to a 1.5 ml centrifuge tube.
(**Note**: If the starting volume is less than 200 µL, adjust the volume to 200 µL with PBS, sterile water, or 0.9% NaCl solution.)
2. Add 250 µL of Buffer VLS, 20 µL of Proteinase K, and 1.0 µL of Carrier RNA. Vortex thoroughly to mix, then incubate at 56°C for 15 minutes.
3. Add 250 µL of absolute ethanol to the lysate and mix thoroughly by pipetting.

Blood:

1. Transfer 10-100 µL of whole blood to a 1.5 ml centrifuge tube and adjust the volume to 200 µL with PBS, sterile water, or 0.9% NaCl solution.
2. Add 250 µL of Buffer VLS, 20 µL of Proteinase K, and 1.0 µL of Carrier RNA. Vortex thoroughly to mix, then incubate at 56°C for 15 minutes.
3. Centrifuge the lysate at 12,000 rpm at room temperature for 3 minutes and transfer supernatant to a new 1.5 ml centrifuge tube.
4. Add 250 µL of absolute ethanol to the supernatant and mix thoroughly by pipetting.

Virus-infected Tissues:

1. Grind 2-10 mg of virus-infected tissue in liquid nitrogen. Add 200 µL of PBS, sterile water, or 0.9% NaCl solution to the ground tissue.
(**Note**: Alternatively, a homogenizer can be used to grind tissue samples. Centrifuge the homogenate at 12,000 rpm at room temperature for 3 minutes and transfer 200 µL of the supernatant to a 1.5 ml centrifuge tube. For example, homogenize 100 mg of tissue sample in 1 ml of PBS.)
2. Immediately add 250 µL of Buffer VLS, 20 µL of Proteinase K, and 1.0 µL of Carrier RNA. Vortex thoroughly to mix, then incubate at 56°C for 15 minutes.

3. Centrifuge the lysate at 12,000 rpm at room temperature for 3 minutes and transfer the supernatant to a new 1.5 ml centrifuge tube.

(**Note:** If a homogenizer is used and centrifugation is performed, this step can be skipped.)

4. Add 250 µL of absolute ethanol to the supernatant and mix thoroughly by pipetting.

7.2 Purification

1. Transfer the above solution to the Virus DNA/RNA Mini Column. Let it stand at room temperature for 2 minutes, then centrifuge at 12,000 rpm at room temperature for 2 minutes, discarding the flow-through.

(**Note**: If the liquid in the column does not completely pass through into the collection tube, increase the centrifuge speed or extend the centrifuge time until all the liquid is transferred.)

2. Add 500 µL of Buffer RWA to the Mini Column, centrifuge at 12,000 rpm at room temperature for 1 minute, and discard the flow-through.

3. Add 750 µL of Buffer RWB to the Mini Column, centrifuge at 12,000 rpm at room temperature for 1 minute, and discard the flow-through.

(**Note**: Ensure that the specified volume of 100% ethanol has been added to Buffer RWB.)

4. Repeat step 3 once.

5. Place the Mini Column in a new 2.0 ml Collection Tube, centrifuge at 12,000 rpm at room temperature for 2 minutes.

(**Note:** Using a new 2.0 ml Collection Tube helps to improve RNA purity.)

6. Place the Mini Column in a new 1.5 ml centrifuge tube, add 20-100 µL of RNase-Free Water to the center of the Mini Column membrane, let it stand at room temperature for 5 minutes, then centrifuge at 12,000 rpm at room temperature for 2 minutes to elute the viral DNA/RNA solution, which can be used for subsequent experiments.

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