ELK Biotechnology

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Viral DNA/RNA Extraction Kit

Catalog No.	Specification	Storage/ Shelf life
EP023-50T	50T	Room temperature/1 year
EP023-200T	200T	Room temperature/1 year

Introduction

The kit is suitable for simple, rapid and efficient separation and purification of DNA/RNA from whole blood, tissue homogenate, swabs, serum, plasma and other acellular body fluids. The unique buffer system enables the viral nucleic acids in the lysate to bind to the silica gel centrifugal adsorbent column in a highly efficient and specific manner, so that the viral nucleic acids obtained are of high purity and stable quality, free of protein, nuclease and other impurities, and can be used in a variety of routine operations, including PCR, fluorescence quantitative PCR and other experiments. It can be used for a variety of routine operations, including PCR, fluorescence quantitative PCR and other experiments.

Kit Components

Component	EP023-50T	EP023-200T	Storage
Buffer RLC	30 mL	120 ml	RT
Buffer PGWT	30 mL	120 ml	RT
Buffer GWT2	30 mL	120 ml	RT
Proteinase K	1.25 mL	1.25 ml*4	-20℃
RNase-Free Water	10 mL	40 ml	RT
Spin Columns DM with Collection Tubes	50	200	RT
RNase-Free Centrifuge Tubes (1.5 mL)	50	200	RT
User Manual	1 copy	1 copy	RT

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Equipment to be Supplied by user

Thermostaticcirculator/vortex mixer

Preparation before use

1. Read this manual carefully before the experiment.

2. Proteinase K needs long-term storage, please place at -20°C.

3. Check whether the Buffer RLC crystallizing or precipitating occurs before use. If there is any crystallization or precipitation, dissolve the Buffer RLC in a water bath at 56°C.

4. Pre-treatment of tissue sample: 20 mg tissue sample is put into 1.5 mL centrifuge tube (prepared by oneself), and 500 µL Buffer RLC was added. After the tissue homogenizer is broken, centrifuge at 12000 rpm (~13400 ×g) for 1 minute, and 200 µL supernatant is taken as sample.

Protocol

1. Take 1.5 mL centrifuge tube (prepared by oneself), add 500 µL Buffer RLC, 200 µL sample, and 20 µL Proteinase K, vortex for 5 s, and then place at room temperature and shake at 1200 rpm for 10 min.

Note: For wet swab samples, take 200 µL for extraction after sufficiently shaking and mixing. For dry swab samples, soak in 400 µL saline, mix well with shaking and leave for 5 min, centrifuge at 12,000 rpm for 1 min, then take 200 µL for extraction.

2. For transient centrifugation, add the solution obtained in step 1 to the Spin Columns DM that has been loaded into the collection tube. Centrifuge at 12,000 rpm (~13,400 ×g) for 1 min, discard the waste solution, and place the column back into the collection tube.

3. Add 500 µL Buffer PGWT to the adsorbent column, centrifuge at 12,000 rpm for 1 min, discard the waste solution, and put the adsorbent column back into the collection tube.

4. Add 500 µL Buffer GWT2 to the adsorbent column, centrifuge at 12,000 rpm for 1 min, discard the waste solution, and put the adsorbent column back into the collection tube.

5. Centrifuge at 12,000 rpm for 2 min and discard the waste solution. Place the adsorption column at room temperature for 2 min and allow to dry.

6. Place the adsorption column in a new RNase-Free Centrifuge Tube, add 40-100 μL of RNase-Free Water to the center of the adsorption column membrane overhanging the column. It is placed at room temperature for 2 min and centrifuged at 12,000 rpm for 1 min to collect nucleic acid solution. Store at -80°C for long term.

Detection of RNA purity and concentration

Integrity: RNA can be detected by ordinary agarose gel electrophoresis (electrophoresis conditions: gel concentration 1.2%; 1 × TAE running buffer; 120V, 20 min). Since 70% - 80% of the RNA in the cells is rRNA, you should see very obvious rRNA bands under UV after electrophoresis. The amount of 28S rRNA is about twice that of 18S rRNA, indicating that the integrity of the RNA is better.

Purity: The OD_{260} / OD_{280} ratio is an indicator of the degree of protein contamination. High-quality RNA, OD_{260} / OD_{280} readings are between 1.8-2.1, and a ratio of 2.0 is a hallmark of high-quality RNA. The OD_{260} / OD_{280} reading is affected by the pH of the solution used in the assay. The same RNA sample, assuming an OD_{260} / OD_{280} reading of 1.8-2.1 measured in a 10 mM Tris, pH 7.5 solution, may read between 1.5-1.9 in an aqueous solution, but this does not indicate RNA Impure.

Concentration: Take a certain amount of RNA extract, dilute n times with RNase-Free ddH_2O, zero the spectrophotometer with RNase-Free ddH_2O, take the diluted solution for OD_{260} / OD_{280} measurement, and calculate the RNA concentration according to the following formula: Final concentration (ng /μl) = (OD_{260}) × (dilution multiple n) × 40.