

Spin Pathogen DNA/RNA Kit

Cat.No.: HYC427

Product Description

This kit is designed for total pathogen RNA and DNA extraction from serum, plasma, milk, body fluids, culture medium, tissue homogenates, swabs and other specimens. It recovers viral DNA/RNA, bacterial DNA/RNA, fungal DNA/RNA, as well as host cellular total DNA and RNA. Built on silica-column purification technology, this workflow eliminates toxic phenol-chloroform extraction and time-consuming ethanol precipitation steps. Purified RNA is directly compatible with RT-PCR, Northern blotting, viral detection and other downstream assays. Purified DNA can be directly applied to conventional PCR, real-time quantitative PCR, viral detection and related analyses.

Components

Components	50 T
Homogenizer tubes C	50
Viral Micro Columns	50
2ml Collection Tubes	50
Proteinase K Solution	1.2 mL
Buffer MLBN	30 mL
Buffer VHB*	13 mL
Buffer RW2*	20 mL
Nuclease Free Water	10 mL

Storage

This product can be stored at room temperature (15–25°C) for 18 months.

Instructions

Preparations

- Absolute Ethanol
- Centrifuge tubes
- RNase-free pipette tips

- Prior to use, dilute Buffer RW2 with absolute ethanol and store at room temperature.
- Prior to use, dilute Buffer VHB with absolute ethanol and store at room temperature.

Protocol

1. Add 20 μ L Proteinase K and 400–500 μ L test sample (whole blood, serum, plasma, culture medium, secretion, swab eluate, tissue homogenate, etc.) into a 2 mL Homogenizer Tube C, then tightly cap the tube.
 - a. **Tissue samples:** Cut tissues into small fragments, transfer 10–50 mg tissue into Homogenizer Tube C, then add 20 μ L Proteinase K and 500 μ L Buffer TE, Buffer PBS or normal saline.
 - b. **Large-volume samples:** Transfer 1.5–5.0 mL plasma, ascites or effusion into a 2–5 mL centrifuge tube. Centrifuge at $13,000 \times g$ for 10 min to pellet microorganisms and cells. Discard supernatant, retain 500 μ L residual liquid or cell pellet, and vortex to resuspend for subsequent use.
 - c. **Sputum samples:** Take an appropriate volume of sputum, add 2–4 volumes of 0.1% DTT for liquefaction. Centrifuge at $13,000 \times g$ for 10 min to pellet microorganisms and cells, discard supernatant, retain 500 μ L residual liquid or pellet, and vortex to resuspend before use.
2. Perform bead-beating lysis using a vortex mixer or bead homogenizer per laboratory equipment availability:
 - a. Vortex mixer: Secure the tube on the mixer, vortex at maximum speed for 5–10 min.
 - b. PowerLyzer bead homogenizer: Run at 2000 rpm for 30 sec, pause for 30 sec, then repeat at 2000 rpm for another 30 sec.
 - c. FastPrep24 bead homogenizer: Run at 5 m/s for 30 sec, pause for 30 sec, then repeat at 5 m/s for another 30 sec.
 - d. TissueLyser II bead homogenizer: Run at 25 Hz for 5 min, reposition the rack, then run again at 25 Hz for another 5 min.
3. Incubate the sample at room temperature or 55 °C for 10 min to complete further digestion, then centrifuge at $12,000 \times g$ for 1 min. This centrifugation step is not required for serum, plasma, swab eluates and similar specimens.
4. Transfer 250 μ L homogenate into a 1.5 mL centrifuge tube, add 500 μ L Buffer MLB, and vortex for 5–10 sec to mix thoroughly.
5. Fit the Viral Micro Column onto a 2 mL collection tube. Load the entire lysate onto the column, then centrifuge at $10,000 \times g$ for 30–60 sec.

6. Discard flow-through and reattach the column to the collection tube. Add 500 μL ethanol-diluted Buffer VHB to the column, then centrifuge at $10,000 \times g$ for 30–60 sec.
7. Discard flow-through and reattach the column to the collection tube. Add 500 μL ethanol-diluted Buffer RW2 to the column, then centrifuge at $10,000 \times g$ for 30–60 sec.
8. Discard flow-through and reattach the column to the collection tube. Add another 500 μL ethanol-diluted Buffer RW2 to the column, then centrifuge at $10,000 \times g$ for 30–60 sec.
9. Discard flow-through and reattach the column to the collection tube. Centrifuge the empty column at $13,000 \times g$ for 3 min to dry residual liquid.
10. Transfer the dried column into a new 1.5 mL centrifuge tube. Load 30–100 μL Nuclease-Free Water directly onto the center of the silica membrane, incubate for 2 min, then centrifuge at $13,000 \times g$ for 1 min.
11. Discard the column and store purified DNA/RNA at $-80\text{ }^{\circ}\text{C}$.