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Biochemicals, Buffers and Reagents

Viral DNA/RNA Extraction Kit

Product No: BIO-9010-50

The Viral DNA/RNA Extraction Kit allows rapid extraction of viral DNA/RNA (nucleic acid) from serum, plasma, body fluids, cell cultured supernatant and viral transport medium swabs. It is a column-based extraction method that utilizes silica membrane spin column technology. The procedure involves lysis of viral cells, optimum binding of viral nucleic acid onto the silica membrane, washing and finally eluting viral nucleic acid from the silica membrane. This kit has been optimized to isolate viral nucleic acid. In this kit, inert carrier [poly-A] powder is included to enhance the binding of viral nucleic acid onto silica membrane to improve its binding efficiency. It is an ideal kit to isolate low viral copies from 10^1 to 10^9 copies of viral nucleic acid. With handling time less than 30 minutes for each preparation, the purified viral nucleic acid is ideal for RT-PCR and qRT-PCR applications directly.

For Research Use Only. Not for use in diagnostic procedures.

Kit Contents

No	Product	BIO-9010-50	Preparation Before Use	Storage
1	VNA Lysis Buffer	21 mL	-	Ambient temperature, 20°C to 25°C
2	BS Buffer **(Concentrated)**	4 mL	Add 30 mL Absolute Ethanol (95-99%)	
3	WBA Buffer	21 mL	-	
4	WBB Buffer **(Concentrated)**	8 mL	Add 32 mL Absolute Ethanol (95-99%)	
5	Inert Carrier powder [Poly-A]	1 mg	Add 1 mL of RNase-free water. Aliquot into several tubes, avoid freeze thaw > 3 times	-20°C
6	RNase-free Water	1.5 mL x 3	-	Ambient temperature, 20°C to 25°C
7	VNA Column	50 pieces	-	
8	2 mL Collection Tubes	100 pieces	-	

Product Specification

Sample size: 200 µL serum, plasma, body fluids, cell cultured supernatant or viral transport medium swaps

Format: Silica membrane spin-column

Operation time: < 30 minutes

Elution Volume: 30 to 50 µL

Storage: Ship at ambient temperature, 20°C to 25°C.
The dissolved inert carrier solution at 1mg/ mL must store at -20°C.

Materials Supplied by Users

- ✓ Micro-centrifuge (non-refrigerated) with minimum speed of 14,000 ×g
- ✓ Absolute ethanol (95-99 %)
- ✓ Sterile nuclease-free 1.5 mL micro-centrifuge tubes, 100 tubes
- ✓ Sterile nuclease-free pipette and pipette tips

Precautions for Users

- ✓ Always wear a lab coat, disposable gloves, anti-fog protective goggles and surgical mask.
- ✓ For viral sample that is potentially contagious, suitable protective PPE lab coats and face shield must be equipped. The extraction works should be conducted in BSL2 or higher grade, which is according to interim guidelines of laboratories.

Protocol

Lysis	1. Prepare the **Lysis Buffer** according to the number of reactions needed in ***Table 1***. For each sample, 400uL of Lysis Buffer is required. 2. Add **200 µL** of **sample** into self-prepared 1.5 mL micro-centrifuge tube. * [If sample is less than 200 µL, adjust the sample volume to 200 µL with 1xPBS] 3. Transfer **400 µL** of **Lysis Buffer**, which prepared from Step 1 into each sample. 4. Vortex for **5 seconds**. Then incubate at room temperature for **10 minutes.**
DNA/ RNA Binding	5. Add **450 µL** of **BS Buffer** (make sure absolute ethanol is added before use) to the sample lysate and mix vigorously. Centrifuge briefly to collect any liquid droplets from the lid. 6. Insert VNA column to a new 2 mL collection tube. Add not more than **600 µL of lysate** to VNA column and centrifuge at 14,000 to 16,000 ×g for **1 minute**. Discard the flow-through. 7. Transfer the **remaining lysate** to VNA column. Again, centrifuge at 14,000 to 16,000 ×g for **1 minute** and discard the flow-through.
Wash	8. Transfer the VNA column to a new 2 mL collection tube. 9. Add **400 µL** of **WBA buffer** into the column and centrifuge at 14,000 to 16, 000 ×g for **1 minute**. Discard the flow-through. 10. Add **600 µL** of **WBB buffer** (make sure absolute ethanol is added before use) and centrifuge at 14,000 to 16,000 ×g for **1 minute**. Discard the flow-through. 11. Centrifuge again at 14,000 to 16,000 ×g for **3 minutes** to dry the column.
Elution	12. Place the dried VNA column into another new set of self-prepared 1.5 mL micro-centrifuge tube. 13. Add **30 to 50 µL** of **RNase-free water** to the center to the VNA column matrix. 14. Let it stand for **3 minutes** and centrifuge at 14,000 to 16,000 ×g for **1 minute.**

Table 1: Preparation of Lysis Buffer using Inert Carrier & VNA Buffer

Number of Preps	Volume of Inert Carrier Solution (1mg/mL) in µL	Volume of VNA Buffer in µL
1	1	400
2	2	800
3	3	1,200
4	4	1,600
5	5	2,000
6	6	2,400
7	7	2,800
8	8	3,200
9	9	3,600
10	10	4,000

Note: The mixture of inert carrier with VNA Buffer is stable at 2°C to 8°C for 48 hours. If precipitation forms, warm the mixture at 80°C to re-dissolve the precipitants. Do not warm for more than 5 minutes.

Troubleshooting

Problems	**Reasons**
Low yield	▪ Inert carrier degrades due to frequent thaw-freeze. ▪ Sample not lysed completely due to excess amount of sample added. ▪ Carryover ethanol during Step no. 12 before elution.
DNA/RNA degradation	▪ RNase/ DNase contamination at work place.

Product Ordering Information

Product Name	**Packaging Size**	**Product Number**
Viral DNA/RNA Extraction Kit	50 preps	BIO-9010-50
1X Phosphate Buffered Saline (PBS), Ultra-Pure Grade,	500 mL	BUF-2040-1X500mL
1X Phosphate Buffered Saline (PBS), Ultra-Pure Grade, 1L	1 L	BUF-2040-1X1L