PrimeWay Viral DNA/RNA Extraction Kit (KIT-9010)

Sample Types

- Serum
- Plasma
- Viral Transport Medium Swabs
- Supernatant/body fluid of viral infected cells

PrimeWay Viral DNA/RNA Extraction Kit

Product No: KIT-9010

PrimeWay Viral DNA/RNA Extraction Kit allows rapid extraction of viral nucleic acid from serum, plasma, supernatant or body fluid of viral infected cell, 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 by improving its binding efficiency. It is an ideal kit to isolate low viral copies from 10E1 to 10E6 copies/mL of viral nucleic acid. With handling time less than 30 minutes for each preparation, the purified viral nucleic acid is ideal for qPCR, RT-PCR, RT-qPCR & Next Generation Sequencing applications.

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

Kit Contents

No	Product	KIT-9010-10 10 preps	KIT-9010-50 50 preps	KIT-9010-250 250 preps
1	Inert Carrier [Poly-A] powder	1 mg	1 mg	1 mg
2	VNA Buffer	5 mL	21 mL	110 mL
3	BS Buffer	1 mL	4 mL	16 mL
4	WBA Buffer	5 mL	21 mL	110 mL
5	WBB Buffer	2 mL	8 mL	40 mL
6	RNase-free Water	1.6 mL	4 mL	15 mL
7	PrimeWay VNA Column	10 pcs	50 pcs	5 x 50 pcs
8	Collection Tubes	2 x 10 pcs	2 x 50 pcs	10 x 50 pcs

Storage

This kit should be stored at room temperature (21 – 25 °C). For longer storage, store Inert Carrier [Poly-A] powder at - 20 °C.

Product Specification

	KIT-9010
Sample	≤ 200 µL serum, plasma, supernatant or body fluid of viral infected cell, and viral transport medium swabs
Yield	Up to 1 µg
Elution	50 µL
Duration	≤ 20 minutes

Materials Supplied by User

- ✓ Centrifuge, at speed of ≥ 14,000 × *g*
- ✓ Vortex mixer
- ✓ Absolute ethanol (≥ 99.5 %)
- ✓ Sterile nuclease-free 1.5 mL microcentrifuge tubes, 2 tubes/sample
- ✓ Sterile nuclease-free pipette and pipette tips

Precautions for User

- ✓ 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 biosafety level cabinet, which is according to interim guidelines of laboratories.

Before Start

- ✓ Add absolute ethanol (≥ 99.5 %) to BS Buffer and WBB Buffer as below:

P/No	BS Buffer	WBB Buffer
KIT-9060-10	1.8 mL	12 mL
KIT-9060-50	9 mL	80 mL
KIT-9060-250	45 mL	200 mL

- ✓ Prepare Inert Carrier Solution by dissolving the **Inert Carrier [Poly-A] powder** with 1 mL of RNase-free water. Vortex to mix. Aliquot the solution into multiple 1.5 mL microcentrifuge tube, avoid freeze-thaw more than 3 times. Store at -20 ^{o}C.

Protocol

Preparation	I. Prepare the **Lysis Buffer** according to the number of reactions needed in **Table 1**. For each sample, 400 µL Lysis Buffer is required.
Lysis (cell-free samples)	1A. For serum, plasma, supernatant or body fluid of viral infected cell a) Add **200 µL sample** into a new 1.5 mL microcentrifuge tube. ***Note**: If sample is less than 200 µL, top up the sample volume to 200 µL with 1X PBS.* b) Add **400 µL Lysis Buffer**, which prepared from Step IV (Preparation step) into each sample. c) Vortex for 5 seconds. Then incubate at room temperature for 10 minutes.
	OR
Lysis (Swab)	1B. For viral transport medium swabs a) Vortex the swabs in the transport medium for 1 minute. b) Transfer **200 µL of the transport medium** to a new 1.5 mL microcentrifuge tube. c) Add **400 µL Lysis Buffer**, which prepared from Step IV (Preparation step) into each sample. d) Vortex for 5 seconds. Then incubate at room temperature for 10 minutes.

DNA/ RNA Binding	2. Add **450 µL BS Buffer** to the sample lysate and mix vigorously. Centrifuge briefly to collect any liquid droplets from the lid. ***Note**: Make sure that ethanol has been added into the BS Buffer.* 3. Insert a **PrimeWay VNA Column** to a new Collection Tube. Add not more than **600 µL lysate** to **PrimeWay VNA Column** and centrifuge at 14,000 - 16,000 × *g* for 1 minute. 4. Discard the flow-through and place the column back to the Collection Tube. 5. Transfer the remaining lysate to **PrimeWay VNA Column**. Again, centrifuge at 14,000 - 16,000 × *g* for 1 minute and discard the Collection Tube.
Washing	6. Place the **PrimeWay VNA Column** to a new Collection Tube. 7. Add **400 µL WBA buffer** into the column and centrifuge at 14,000 - 16, 000 × *g* for 30 seconds. 8. Discard the flow-through and place the column back to the Collection Tube. 9. Add **600 µL WBB Buffer** and centrifuge at 14,000 - 16,000 × *g* for 30 seconds. Discard the flow-through. ***Note**: Make sure that ethanol has been added into the WBB Buffer.*
Drying	10. Centrifuge again at 14,000 - 16,000 × *g* for 3 minutes to dry the column.
Elution	11. Place the **PrimeWay VNA Column** into a new 1.5 mL microcentrifuge tube. 12. Add **50 µL RNase-free water** to the column membrane. 13. Let the column stand for 3 minutes and centrifuge at 14,000 - 16,000 × *g* for 1 minute.

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

Number of Preps	Volume of Inert Carrier Solution (1 mg/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 Solution 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 Guidelines

Problems	Possible Reasons	Recommended Action
Low yield	Inert Carrier degrades due to frequent freeze-thaw	Aliquot Inert Carrier solution into multiple tubes to avoid frequent freeze-thaw.
	Sample not lysed completely due to excess amount of sample added	Reduce sample input.
	Carryover ethanol during Washing Step before elution	Before Elution, ensure Drying process of the column is done according to the protocol.
DNA/RNA degradation	RNase/DNase contamination at workplace	Sterilize workplace with nuclease decontamination solution before start. Use new set of gloves.

Please contact us at https://base-asia.com/contact/ for more information.

Product Ordering Information

Part Number	Product Description	Remarks
1st BASE BUF-2040-1X500mL	1X Phosphate Buffered Saline (PBS), Ultra-Pure Grade, 500 mL	Sufficient for 2,500 preps
1st BASE CUS-7020-1L	Viral Transport Media (VTM), Biotechnology Grade, 1L.	Sufficient for 5,000 preps