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PrimeWay Viral DNA/RNA Extraction Kit (KIT-9010-50)

Sample Types

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





PrimeWay Viral DNA/RNA Extraction Kit

Product No: KIT-9010-50

PrimeWay Viral DNA/RNA Extraction Kit allows rapid extraction of viral DNA/RNA (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 to improve its binding efficiency. It is an ideal kit to isolate low viral copies from 10^1 – 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 RT-qPCR applications directly.

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

Kit Contents

| No | Product | KIT-9010-50 | Storage |
|----|-------------------------------|-------------|---|
| 1 | Inert Carrier powder [Poly-A] | 1 mg | -20 °C (Upon received) Room temperature (21 °C - 25 °C) |
| 2 | VNA Buffer | 21 mL | |
| 3 | BS Buffer | 4 mL | |
| 4 | WBA Buffer | 21 mL | |
| 5 | WBB Buffer | 8 mL | |
| 6 | RNase-free Water | 1.5 mL x 3 | |
| 7 | PrimeWay VNA Column | 50 pcs | |
| 8 | Collection Tubes | 100 pcs | |



Product Specification

| KIT-9010-50 | |
|-------------|--|
| Sample | ≤ 200 µL serum, plasma, supernatant or body fluid of viral infected cell, viral transport medium swabs |
| Elution | 30 - 50 µL |
| Duration | < 30 minutes |
| Storage | Room temperature (21 °C - 25 °C), -20 °C |

Materials Supplied by Users

- ✓ Centrifuge at speed of $\geq 14,000 \times g$
- ✓ Absolute ethanol ($\geq 99.5\%$)
- ✓ Sterile nuclease-free 1.5 mL microcentrifuge tubes, 2 tubes/sample
- ✓ 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 biosafety level cabinet, which is according to interim guidelines of laboratories.



Protocol

| | |
|------------------|--|
| Preparation | <ol style="list-style-type: none"> I. Add 30 mL of absolute ethanol into BS Buffer. Mix well before use. II. Add 32 mL of absolute ethanol into WBB Buffer. Mix well before use. III. Dissolve the Inert Carrier powder [Poly-A] 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. |
| Lysis | <ol style="list-style-type: none"> 1. Prepare the Lysis Buffer according to the number of reactions needed in Table 1. For each sample, 400 μL of Lysis Buffer is required. 2. Add 200 μL of sample into a new 1.5 mL microcentrifuge tube. Note: <i>If sample is less than 200 μL, top up the sample volume to 200 μL with 1X PBS.</i> 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 | <ol style="list-style-type: none"> 5. Add 450 μL of BS Buffer to the sample lysate and mix vigorously. Centrifuge briefly to collect any liquid droplets from the lid. Note: <i>Make sure that ethanol has been added into the BS Buffer.</i> 6. Insert a PrimeWay VNA Column to a new Collection Tube. Add not more than 600 μL of lysate to PrimeWay VNA Column and centrifuge at 14,000 - 16,000 $\times g$ for 1 minute. 7. Discard the flow-through and place the column back to the Collection Tube. 8. Transfer the remaining lysate to PrimeWay VNA Column. Again, centrifuge at 14,000 - 16,000 $\times g$ for 1 minute and discard the Collection Tube. |



| | |
|---------|--|
| Washing | <ol style="list-style-type: none">9. Place the PrimeWay VNA Column to a new Collection Tube.10. Add 400 μL of WBA buffer into the column and centrifuge at 14,000 - 16,000 $\times g$ for 1 minute.11. Discard the flow-through and place the column back to the Collection Tube.12. Add 600 μL of WBB Buffer and centrifuge at 14,000 - 16,000 $\times g$ for 1 minute. Discard the flow-through. Note: <i>Make sure that ethanol has been added into the WBB Buffer.</i>13. Centrifuge again at 14,000 - 16,000 $\times g$ for 3 minutes to dry the column. |
| Elution | <ol style="list-style-type: none">14. Place the PrimeWay VNA Column into a new 1.5 mL microcentrifuge tube.15. Add 30 - 50 μL of RNase-free water to the column membrane.16. Let the column stand for 3 minutes and centrifuge at 14,000 - 16,000 $\times g$ for 1 minute. |



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

| Number of Preps | Volume of Inert Carrier Solution (1mg/mL) in μ L | Volume of VNA Buffer in μ L |
|-----------------|--|---------------------------------|
| 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

| Problems | Reasons |
|---------------------|--|
| Low yield | <ul style="list-style-type: none"> ▪ Inert Carrier degrades due to frequent freeze-thaw. ▪ Sample not lysed completely due to excess amount of sample added. ▪ Carryover ethanol during Step 12 before elution. |
| DNA/RNA degradation | <ul style="list-style-type: none"> ▪ RNase/ DNase contamination at workplace. |

Product Ordering Information

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

Please contact us at <http://www.base-asia.com/find-us> for more information.

