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AuPreP Viral RNA Extraction Kit

PRM74-904LT / PRM74-906LT

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AuPreP Viral RNA Extraction Kit

Name: AuPreP Viral RNA Extraction Kit
Cat. No.: PRM74-904LT / PRM74-906LT
Qty.: 50 Preps / 250 Preps

Product Description:-

The AuPreP Viral RNA Extraction Kit is designed to isolate pure, high quality viral RNA from up to 100mg of a wide variety of plant matrices. It is important to note that product does not isolate only viral RNA, but it has been tested widely in molecular biology labs working on Plant Virus applications.

Kit Components and Storage:

- PRL buffer (50 ml or 250 ml)
- PRW1 buffer (50 ml or 250 ml)
- PRW2 buffer (10 ml or 50 ml)
- Elution buffer (15 ml)
- Spin columns with collection tubes (50 or 250)

Reagents Required but Not Provided:

- Ethanol
- 6-mercaptoethanol (6ME)
- RNase-Free microcentrifuge tubes

Storage Temperature: All components can be stored at ambient temperatures of 25-30°C for up to 1 year unless specifically mentioned. While the product performs best when stored in this range, it can also perform when exposed to higher room temperatures. See the expiration date on the Product Information Label.

Quality Control: In accordance with our ISO-certified Quality Management System, each lot of Viral RNA Extraction Kit is tested against predetermined specifications to ensure consistent product quality.

Application & Principle:

The kit enables isolation of viral RNA from plant matrices for further use in PCR, RT-PCR, qPCR analysis, and a wide variety of sensitive downstream applications. Fresh or frozen plant tissues are homogenized using standard methods, such as mortar and pestle with liquid nitrogen. After cell lysis, the RNA is captured on a silica-membrane spin filter. The RNA bound to the filter is washed to remove contaminants. Finally, the RNA is recovered in an elution buffer and the eluted RNA is ready for downstream applications.

Before You Begin:

- **Note:** Add 10 µl β-mercaptoethanol (βME) to 1 ml PRL buffer (per sample) immediately before use.
- For sample preparation, standard methods, including a high-powered bead beater, a rotor-stator homogenizer or a mortar and pestle with liquid nitrogen are all acceptable methods of preparation.
- Add 95-100% ethanol to Buffer PRW2 before use (as mentioned below)

For PRM74-904LT: Add 40 ml of ethanol to make final volume to 50 ml
For PRM74-906LT: Add 200 ml of ethanol to make final volume to 250 ml

Note: *RNA yields from plant tissues can vary considerably based on the age and type of tissue and how the samples were stored.*

Recommended Protocol

Step 1: Sample Preparation

1. Fresh or frozen plant tissue samples need to be homogenized using standard methods. For example, grind up to 100 mg of plant tissue in liquid nitrogen until a fine powder is achieved. Transfer the
powder to a 2 ml microcentrifuge tube (RNase-free microcentrifuge tubes are recommended). After briefly allowing the liquid nitrogen to fully evaporate, immediately proceed to the next step.

2. Add 1 ml Buffer PRL to the prepared tissue powder in the microcentrifuge tube. Vortex vigorously using a vortex mixer. Incubate at 70°C for 10 minutes, mixing intermittently every 3 minutes. Centrifuge at 14000-16000 x g for 5 minutes.

3. Carefully, transfer 450 µl of supernatant to a 2 ml microcentrifuge tube (not provided) without disturbing the cell-debris pellet. Add 225 µl (0.5 volume) of ethanol (95-100%) and mix gently by pipetting.

Step 2: RNA Capture

4. Transfer entire contents (usually 650 µl, with precipitate if any), to a spin column within a 2 ml collection tube. Centrifuge for 30 seconds at ≥8000 x g (≥10,000 rpm). Discard the flow-through.
If the entire mixture does not pass through the spin column, repeat the centrifugation, and increase the centrifuge time for future preps of similar tissue type.

Step 3: Column Wash

5. Add 700 µl Buffer PRW1 to the spin column. Centrifuge for 30 s at ≥8000 x g. Discard the flowthrough.

6. Add 500 µl Buffer PRW2 to the spin column. Centrifuge for 30 s at ≥8000 x g. Discard the flowthrough.

7. Add 500 µl Buffer PRW2 to the spin column. Centrifuge for 2 min at ≥8000 x g. Centrifuge at full speed for 1 min to dry the spin column membrane.

Step 4: RNA Elution

8. Place the spin column in a new 1.5 ml collection tube (not provided). Add 30–50 µl elution buffer directly to the spin column membrane, depending on desired concentration. Centrifuge for 1 min at ≥8000 x g to elute the RNA.

9. If the expected RNA yield is >30 µg, repeat step 8 using another 30–50 µl of Elution Buffer. Note: If higher RNA concentration is required, repeat the above step using the final eluate. 10. Store RNA at -70°C.

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