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# PHYTORENA
# Plant RNA Isolation Kit

**Cat. No:**

**FPKT034.0025**

**FPKT034.0050**

**FPKT034.0100**

**Contents:**

| Components | 25 preps | 50 preps | 100 preps |
|---|---|---|---|
| LR buffer | 20 ml | 40 ml | 80 ml |
| PW buffer | 6 ml | 12 ml | 12 ml * 2 |
| ER buffer | 2 ml | 2 ml | 2 ml |
| Column and collection tubes | 25 each | 50 each | 100 each |

## Kit storage:

⚠ This kit should be stored at room temperature.
If properly stored, all kit components are stable until the expiration date printed on the label.

## Additional Equipment and Reagent required

- Make sure everything is RNase-free when handling RNA.
- Absolute ethanol
- ethanol 70%
- Chloroform
- Standard tabletop microcentrifuge capable of 13,000 x *g* centrifugal force
- Microcentrifuge tubes, 1.5 ml, sterile

## Application

The High Pure RNA Isolation Kit is designed for the purification of total RNA from Plants. RNA is suited for other techniques like northern blotting, RNase protection and primer extension. The procedure is optimized to achieve reliable results within Approximately **1 hour (24 samples simultaneously).**

## Handling Requirements and Safety Information

⚠ All solutions are clear and should not be used when precipitates have formed. Warm the solutions at +15 to +25°C or in a 37°C water bath until the precipitates have dissolved.

⚠ LR Buffer and contain guanidinium hydrochloride which is an irritant.

⚠ Do not allow LR Buffer to touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water.

⚠ If you spill the reagent, dilute the spill with water before wiping it up.

⚠ Do not use any modified ethanol.

⚠ Do not pool reagents from different lot numbers.

⚠ Immediately after usage, close all bottles in order to avoid leakage, varying buffer concentrations or buffer conditions.

⚠ After first opening store all bottles in an upright position.

⚠ Do not allow the LR Buffer to mix with sodium hypochlorite found in commercial bleach solutions. This mixture can produce a highly toxic gas.

⚠ Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.

⚠ Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipets and nuclease free pipet tips only, to remove aliquots from reagent bottles.

## preparation procedure:

**Working Solution Preparation**

FPKT034.0025-25 prep

⚠ **Add 24 ml absolute ethanol to PW Bottle.**

FPKT034.0050-50 prep

⚠ **Add 48 ml absolute ethanol to PW Bottle**

FPKT034.0100-100 prep

⚠ **Add 48 ml absolute ethanol to each PW Bottle.**

⚠Please note that the Ethanol concentration of a Washing Buffer may decrease during long term storage resulting in a drop-down of the final RNA yield.

⚠ before starting Incubate the **ER buffer at 55 to 65 ° C** until the end of the protocol to obtain the maximum yields.

## Protocol

**1. Pipette 800 µl of Plant LR Solution**, into 1.5 mL microcentrifuge tube (not provided). Weigh the plant tissue - use **50-100 mg** of fresh or frozen tissue; up to **20 mg** of lyophilized tissue.

**Grind the material using one of the following methods:**

**a) Mortar and Pestle**

Place up to 100 mg of plant tissue into liquid nitrogen and grind thoroughly with a mortar and pestle.

**b) Grinding mill**

Place up to 100 mg of tissue into a vial containing stainless steel beads. The vial and beads should be precooled with liquid nitrogen. The setup of the mechanical disruption depends on the tissue type.

Immediately transfer the tissue powder into a 1.5 mL microcentrifuge tube
containing 800 µl of Plant LR Solution. Vortex for 10-20 s to mix thoroughly **Incubate for 15 min at room temperature.**

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**2. Add 200 µl Chloroform** and shake it for 10-20 s to mix thoroughly **Incubate for 3 min at room temperature.**

**3. Centrifuge at full speed and 4°C for 12 min.**

**4. transfer 400 µl of the upper phase** into a new 1.5ml tube. **Add 400 µl** Absolute ethanol and mix well generously.

**5.** transfer the sample to a High Pure Filter Tube. **Centrifuge at full speed for 1 min.**

**6.** Discard the flow through add **700 µl PW Buffer** and **Centrifuge at full speed for 1 min**.

**7. (Optional for more pure RNA)** Discard the flow through add **500 µl PW Buffer** and **Centrifuge at full speed for 1 min.**

**8.** Leave the tube assembly in the centrifuge and spin it for **3 min at maximum speed** (approximately 14,000 rpm) to remove any residual PW Buffer.

**9.** Discard the Collection Tube and insert the Filter Tube into a clean, sterile RNase free and DNase free **1.5 ml microcentrifuge tube.**

**10. Add 35 µl ER Buffer** to the upper of the Filter Tube. Centrifuge the tube assembly for **1 min** at maximum speed. The microcentrifuge tube now contains the eluted Cell RNA. Either use the eluted RNA directly in RT.PCR or store the eluted RNA at –70°C for later analysis.