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RNrich Plant Kit

Introduction

The **RNrich Plant Kit** is designed for RNA extraction from different sources of plants such as leaf or seed. The quality of extracted RNA can be varied, depending on the samples used. The kit is intended for downstream application of molecular biology.

RNrich Plant Kit components*	Cat. No. AEPRX-1105-050
A solution**	25 ml
B Solution	15 ml
C Solution	15 ml
Wash Buffer(conc.) **	50 ml
Elution Buffer	3 ml
Manual	1

* 1.5 ml micro-centrifuge tube, **molecular biology grade water** and **ethanol absolute** are needed but are not included.

** Please refer to reminder, **A solution** and **Wash Buffer Preparation** before using this kit.

Caution

The components contain irritants. During operation, always wear a lab coat, disposable gloves, and protective goggles.

Reminder

Pre-set heater block at **56°C**.

During RNA extraction, **never** open the microtube cap outside the laminar hood.

A Solution preparation

Add **22 ml** of molecular biology grade water to **A Solution**, and shake it well.

Note: mark the check box on the bottle.

Note: With **1 M NaOH** adjust the pH to 8.

Note: For the best results, the prepared **A Solution** should be used immediately. Prepared **A Solution** can be stored for up to one month at **4°C**.

Wash Buffer Preparation

Add **35 ml molecular grade absolute ethanol** **Wash Buffer** bottle before first use and mark the check box on the bottle.

PROTOCOL

Step 1: Sample Preparation

- Cut off **50 mg** (up to 200 mg) of plant tissue.
- **Grind** the sample to a fine powder and transfer it to a 1.5 ml micro-centrifuge tube.
Note: Some plant samples need liquid nitrogen and/or washed and autoclaved sand to be ground sufficiently.

Step 2: Lysis

- **Note:** Prepare **A Solution** immediately prior to use.
- Add **500 µl of pre-warmed A Solution** into the sample tube and mix by vortex.
- **Incubate at 56°C** for **90** minutes. During incubation, vortex vigorously the tube every **30** minutes.
Note: Incubation time could be varied from 30 minutes to overnight for different plant samples.
- Add **300 µl of B Solution** and mix by vortex for 30 seconds.
- Keep at **RT** for **10** minutes.
- Centrifuge for **15** minutes at **12000 g**.
- Carefully transfer about **300µl of supernatant** to a new 1.5 micro-centrifuge tube and do not disturb the phases.
Note: The amount of supernatant phase varies.

Step 3: RNA Precipitation

- Add **300 µl of C Solution** to sample tube and mix by **invert** for 15 times.
- Incubate the samples at **-20°C** for 90 minutes.
Note: In sample with **low amount** of RNA, incubation time can be up to **overnight**.
- Centrifuge at **12000 g** and **4 °C** for **15** minutes.
- **Decant** the supernatant into waste.
Note: While decanting, be careful not to lose the RNA pellet.

Step 4: Wash

- Add **500 µl of Wash Buffer** to the sample tube, invert for 10 times and then centrifuge at **12000 g and 4 °C** for **15** minutes.
- **Decant** the supernatant into waste.
- Add **500 µl of Wash Buffer** to the sample tube, invert for 10 times and then centrifuge at **12000 g and 4 °C** for **15** minutes.
- **Decant** the supernatant into waste, and let stand until dry.

Step 5: RNA Elution

- Add **50 µl of Elution buffer** to the sample tube.
- Allow to re-suspend the RNA for **5** minutes at RT, and then centrifuge at **10000rpm** for 1 minute.
Note: Store RNA at **-20°C**.

