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BioVision
BioVision Incorporated

EasyRNA™ Plant RNA Mini Kit

(Cat# K1374-50, -250; Store at RT)

I. Introduction:
BioVision's EasyRNA™ Plant RNA Mini Kit provides an easy and fast method for isolating total RNA from plant tissues within 30 min. Only trace genomic DNA exists in the purified RNA, which can be eliminated by DNase I treatment, when it is necessary.

II. Sample Type: For fast and efficient isolation of total RNA from plant tissues within 30 min.

III. Kit Contents:

	K1374-50	K1374-250	
Components	**50 Preparations**	**250 Preparations**	**Part Number**
Buffer PLY	28 mL	130 mL	K1374-XX-1
Buffer XL	35 mL	130 mL	K1374-XX-2
Buffer RB	30 mL	130 mL	K1374-XX-3
Buffer P2	12 mL	25 mL	K1374-XX-4
RNA Wash Buffer*	12 mL	50 mL	K1374-XX-5
DEPC-Treated ddH$_2$O	10 mL	30 mL	K1374-XX-6
RNA Columns	50	250	K1374-XX-7
Homogenization Columns	50	250	K1374-XX-8

**Add 48 mL (K1374-50) or 200 mL (K1374-250) 100% ethanol to RNA Wash Buffer before use.*

IV. User Supplied Reagents and Equipment:

- Tabletop microcentrifuge and 1.5 mL sterile tubes
- Vacuum manifold if use vacuum protocol
- 100% ethanol; 70% ethanol
- β-mercaptoethanol
- **Optional:** DNase I, DNase Buffer

V. Shipment and Storage:
All the reagents are shipped at room temperature. DNase I (optional) should be stored at -20°C. All other components can be stored at room temperature. All kit components are guaranteed for 12 months from the date of purchasing.

VI. Reagent Preparation and Storage Conditions:

- Prepare all components and get all necessary materials ready by examining the protocol and become familiar with each step.
- Determine the volume of Buffer PLY to be used. Add 10 µL of β-mercaptoethanol per 1 ml Buffer PLY.
- Buffer LY may form precipitates below RT. Warm at 37°C to dissolve before use.
- Add 48 mL (K1374-50) or 200 mL (K1374-250) 100% ethanol to RNA Wash Buffer before use.

VII. Protocol for Extracting Total RNA from Plant Tissue:

1. Weigh **30-100 mg** plant tissue in a 2 mL tube. Freeze the plant tissue in liquid nitrogen and grind using a rotor starter.
2. Transfer **500 µL Buffer LY** to the tube containing the plant tissue immediately. Grind using a rotor starter again. ***Ensure that β--mercaptoethanol has been added before use.***
3. Transfer the sample to a **Homogenization Column** and centrifuge the lysate for 5 min at 14,000 rpm. Transfer the cleared lysate to a clean RNase free vial without disturbing the cell-debris pellet into a collection tube
4. Add **1 volume of 70% ethanol** to the lysate (for example: 450 µL 70% ethanol for 450 µL lysate). Mix well by vortexing briefly.
5. Transfer the solution into a RNA column and centrifuge at 12,000 rpm for 1 min. Discard the collection tube with the flow-through and put the column back to a new collection tube.
6. Add **400 µL Buffer RB** to the column and centrifuge at 12,000 rpm for 1 min. Discard the flow-through.
7. Add another **500 µL RNA Wash Buffer** to the column and centrifuge at 12,000 rpm for 30 s. *Ensure that ethanol has been added to RNA Wash Buffer before use. Discard the flow-through.
8. Add another 600 µL RNA Wash Buffer to the column and centrifuge at 13,000 rpm for 30 s. Discard the flow-through and collection tube, put the column, with the lid open, back to a new collection tube.
9. Centrifuge at 14,000 rpm for 1-2 min. Discard the flow-through. *Note: The residual ethanol will be removed more efficiently with the column lid open during centrifugation.*
10. Place the column to a RNase-free 1.5 mL tube, add **50-100 µL DEPC-treated ddH$_2$O** to the column and centrifuge at 13,000 rpm for 1 min. The RNA is in the flow-through liquid. Store the RNA solution at -20°C.

Note: It is highly recommended that RNA quality be determined before downstream applications. The quality of RNA can be assessed by denatured agarose gel electrophoresis with the ethidium bromide staining. Several sharp bands should appear on the gel including 28S and 18S ribosomal RNA bands as well as certain populations of mRNA and bands. If these bands smear towards lower molecular weight RNAs, then the RNA has undergone major degradation during preparation, handling or storage, RNA molecule less than 200 bases in length do not efficiently bind to the RNA column. An A_{260}/A_{280} ratio of 1.8-2.0 corresponds to 90-100% pure nucleic acid.

FOR RESEARCH USE ONLY! Not to be used on humans.