Data sheet

Plant RNA Purification Kit

Cat. No: AN0100 (50 reactions)
Cat. No: AN0102(100 reactions)

Description

Plant RNA Purification Kit offers a rapid and convenient method for purification of total RNA from a variety of plant tissue. The kit is based in nucleic acid ability to bind silica in the presence of high concentrations of chaotropic salts. This system has no need for phenol/ chloroform extraction and centrifugation with CsCl gradients or precipitation with LiCl. Eluted purified RNA is ready for use in a variety of downstream applications: real-time RT-PCR, Northern Blotting, cDNA library construction, etc.

Features

- **High yields**: up tp 30µg total RNA from young leaves.
- **Ready to use** RNA.
- **Just a few minutes** procedure (about 30 min).
- **Mini format**

Quality Certifications

Total RNA is isolated from a 100 mg young leaf sample. Purified RNA is quantified using a spectrophotometer with a typical yield of more than 10µg of total RNA and an A260nm/A280nm ratio of 1.9-2.1. Quality is further checked by agarose gel electrophoresis.

Kit Storage

Plant RNA Purification Kit should be stored at room temperature (15–25°C) for up to 12 months without any reduction in performance. Store DNase I Solution at 4°C.

Kit Components	(Reactions)	
Item	50	100
RNAprep spin columns	50	100
Collection tubes (2 mL)	100	200
Filter column	50	100
L1 Buffer *	30 ml	60 ml
L2 Buffer *	30 ml	60 ml
WB1 Buffer	40 ml	80 ml
WB2 Buffer**	20 ml	40 ml
RNase-free water	10 ml	10 ml
DNase Solution (1U/µl)	1.5 ml	2x1.5 ml

Note

*Various plant species contain different metabolites such as polysaccharides, polyphenols, and proteins. The standard protocol uses **L1 Buffer** for lysis of most common plant species. **L2 Buffer** is provided with the kit to ensure efficient cell lysis of plant species with high polysaccharide content. Before beginning the lysis and homogenization steps, prepare a fresh amount of **L1 Buffer** (**L2 Buffer**) containing 1% 2-mercaptoethanol (β-ME) [Not included] for each purification procedure. Add 10 µL β-ME for each 1 mL Lysis Buffer.
β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing.

**Add absolute ethanol (see the bottle label for volume) to the WB2 Buffer prior to initial use.

Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water.

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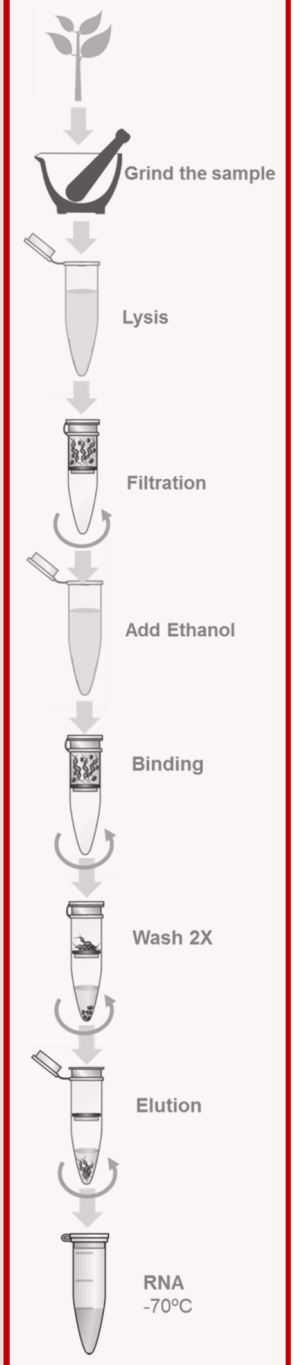
DETAILED PROTOCOL

1. Cut the plant samples and weight them (up to 100mg). Immediately after doing so, place them inside a mortar with liquid nitrogen.
2. Grind the sample under liquid nitrogen to a fine powder.
3. Transfer the sample powder to a 1.5 microcentrifuge tube.
4. Add 500 µL of **L1 Buffer** (or **L2 Buffer**) and mix by vortex vigorously. *Ensure that **β-mercaptoethanol** is added to **L1 Buffer** (or **L2 Buffer**) before use.*
5. Incubate at room temperature for 5 minute.
6. Place a **Filter Column** in a 2 ml **Collection tube** and transfer the sample mixture to the column.
7. Centrifuge at full speed for 1 minute.
8. Carefully transfer the clarified filtrate to a new 1.5 ml microcentrifuge tube.
9. Add 1 volume of 70% ethanol to the clarified lysate and mix vigorously by vortexing.
10. Apply the total volume (usually 700 µl) from step 9 to the **RNAprep spin column** by decanting or pipetting.
11. Centrifuge at full speed (10000g) for 90 seconds. Discard the flow-through.
12. Wash the **RNAprep spin column** by adding 250 **µL WB1** and centrifuging at 10000 g for 90 seconds. Discard the flow-through.
13. Place the **RNAprep spin column** in a **collection tube** and add 60 µL of RNase-free **DNase I solution** (0.5U/µl) to the centre of the column matrix. Let stand for 15 minute at room temperature.
14. Add 500 µl of **WB1** and centrifuge at full speed for 30 seconds. Discard the flow-through.
15. Add 750 µl of **WB2** and centrifuge at full speed for one minute. Discard the flow-through.
16. Repeat the step 15.
17. Again Centrifuge at full speed for 3 minute. This step helps to dry the **RNAprep spin column**.
18. Place the **RNAprep column** into a new, labelled 1.5 microcentrifuge tube and pipet 50-60µl of **RNase-free Water** directly into the. Close the cap and incubate for 1 minute at room temperature.
19. Centrifuge at full speed for 1 minute to elute RNA.
20. Keep eluted RNA on ice at all times and store at <-70°C.



PRODUCT USE LIMITATION : This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, and is not suitable for administration to humans or animals. Please refer to www.canvaxbiotech.com for the Material Safety Data Sheet of the product.