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DATA SHEET
Version: 03
Revision date: 22/05/2023

1. Identification

Product name **HigherPurity™ Plant RNA Purification Kit**

Cat. No. **AN0100 (50 reactions)**
Cat. No. **AN0102 (100 reactions)**
Cat. No. **AN0102-XL (250 reactions)**

2. Description

HigherPurity™ 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.

3. Kit Contents

Item	Quantity		
	AN0100 (50 rxn)	AN0102 (100 rxn)	AN0102 (250 rxn)
L1 Buffer *	28 ml	55 ml	140 ml
L2 Buffer *	28 ml	55 ml	140 ml
WB1 Buffer	28 ml	55 ml	140 ml
WB2 Buffer**	16 ml	32 ml	80 ml
RNase-free water	5 ml	8 ml	15 ml
RNAprep spin columns	50	100	250
Collection tubes (2 mL)	100	200	500
Filter column	50	100	250

*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 (βME) [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 the volume ethanol (96%-100%) specified [Not included] to WB2 Buffer prior to initial use (see bottle label for volume). After ethanol has been added, mark the bottle to indicate that this step has been completed.

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

4. Storage Specifications

All components can be stored at Room Temperature

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5. Applications

Purified RNA suitable for all common Molecular Biology applications, such as:

- RT-PCR.
- Northern Blotting.

6. 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.

7. 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.

8. Further information

Product Use Limitations	This product is developed, designed, and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Safety Information	When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at www.canvaxbio.com where you can find, view, and print the MSDS for each CANVAX kit.

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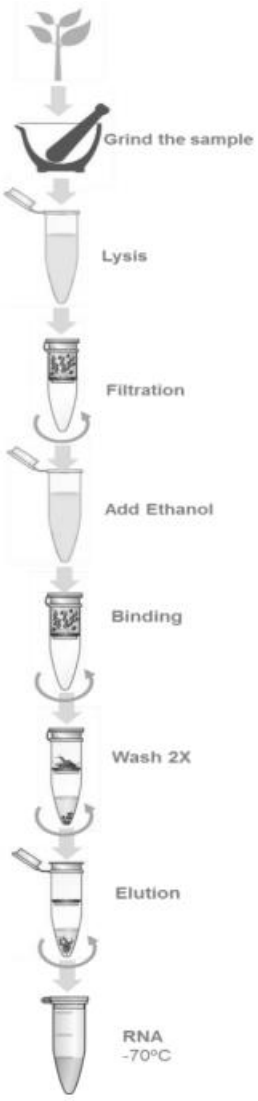
Assay Procedure



1. Cut the plant samples and weight them (up to 100mg). Immediately after doing so, place them inside a mortar with liquid nitrogen. Grind the sample under liquid nitrogen to a fine powder.
2. Transfer the sample powder to a 1.5 microcentrifuge tube [not provided].
3. 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.*
4. Incubate at room temperature for 5 minutes.
5. Place a Filter Column in a 2 ml Collection tube and transfer the sample mixture to the column. Centrifuge at full speed for 1 minute.
6. Carefully transfer the clarified filtrate to a new 1.5 ml microcentrifuge tube [not provided].
7. Add 1 volume of 70% ethanol to the clarified lysate and mix vigorously by vortexing.
8. Apply the total volume (usually 700 µl) from step 7 to the RNAprep spin column by decanting or pipetting.
9. Centrifuge at full speed (10000g) for 90 seconds. Discard the flow-through.
10. [Optional]: To eliminate genomic DNA contamination (Otherwise, proceed to step 11 directly).
    a. Wash the RNAprep spin column by adding 250 µL WB1 and centrifuging at 10000 g for 60 seconds. Discard the flow-through.
    b. Perform optional on-column DNase digestion using the RNase-Free DNase I Set (Ref. EZ0020, not included in kit).
    c. Wash the RNAprep spin column by adding **250 µL WB1** and centrifuging at 10000 g for 60 seconds. Discard the flow-through.
    d. After DNase I treatment, proceed to step 12.
    e. Alternatively, after the RNA extraction procedure, use DNase I to digest the RNA eluate. After inactivating the DNase I by heat treatment, the RNA can be either used directly in the downstream application without further treatment, or repurified using the RNA clean up protocol [DNase I not included].
11. Add **500 µl of WB1** and centrifuge at full speed for 60 seconds. Discard the flow-through.
12. Add **750 µl of WB2** and centrifuge at full speed for one minute. Discard the flow-through.
13. Repeat the step 12.
14. Again, centrifuge at full speed for 3 minutes. This step helps to dry the RNAprep spin column.
15. Place the RNAprep column into a new, labelled 1.5 microcentrifuge tube and pipet **30-50µl of RNase-free Water** directly into the center. Close the cap and incubate for 1 minute at room temperature.
16. Centrifuge at full speed for 1 minute to elute RNA.
17. After extraction place the Elution Tube on ice. For long time storage place the nucleic acids at –80˚C.