WizPrep™ Plant RNA Mini Kit

RUO For Research Use Only

REF W72650

DESCRIPTION

The WizPrep™ Plant RNA Mini Kit provides for rapid isolation of RNA from various plant samples (e.g. leaves, stems, buds, flowers, fruit, seeds, etc.). For purification of total RNA (~25 µg), the WizPrep™ Plant RNA Mini Kit features a specially formulated RNA Lysis Buffer. The Spin Column allows for high-capacity DNA elimination and the subsequent Spin Column efficiently adsorbs total RNA. The RNA is washed and then eluted with DNase/RNase-Free Water. The RNA is suitable for use in various subsequent procedures including RT-PCR. The entire RNA isolation procedure typically takes about 15 minutes.

KIT CONTENTS

Contents	50 prep	150 prep	Storage
RPL1 Buffer	25 ml	80 ml	Room temp.
RPW1 Buffer	40 ml	120 ml	Room temp.
RPW2 Buffer (concentrate)(1)	12 ml	40 ml	Room temp.
DNase/RNase-Free Water	5 ml	20 ml	Room temp.
Spin Columns*	50	150	Room temp.
Filter Columns*	50	150	Room temp.
Instruction Manual	1	1	

(1) : Add absolute ethanol to the RW2 Buffer prior to initial use (see the bottle label for volume).

* All Spin Columns are sterilized by electron beam.

REAGENTS AND EQUIPMENT TO BE SUPPLIED BY USER

- 96-100% ethanol (to prepare RPW2 Buffer)
- 1.5 mL microcentrifuge tubes
- Sterile RNase-free pipette tips and Manual pipettors
- Centrifuge for microcentrifuge tubes
- Equipment for sample disruption and homogenization
- Personal protection equipment (lab coat, gloves, goggles)

KIT SPECIFICATIONS

Parameter	Characteristics
Format	Silca-membrane spin column
Sample materials	< 100 mg tissue
Fragment size	> 200 nt
Typical yield	3-70 ug from 100 mg plant material
$A_{260/280}$	1.9 - 2.1
Elution volume	50 µl
Preparation time	20 min. / 6 prep
Binding capacity	200 ug

QUALITY CONTROL ANALYSIS

In accordance with Wizbiosolutions Inc. ISO 13485-certified Quality Management System, each lot WizPrep™ Plant RNA Mini Kit is tested against predetermined specifications to ensure consistent product quality.

PROTOCOL

To check before start

Before starting, add 48 (200) ml of 100% ethanol to the 12 (40) ml of RPW2 Buffer concentrate.

1. Prepare plant tissue

- Transfer ≤100 mg of plant tissue or 5 x 10^6 plant cells into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample.
- Grind the sample into a fine powder using a pestle in liquid nitrogen.

Note: If stored frozen samples are used, do not allow the samples to thaw before transferring to the liquid nitrogen. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.

2. Lysis

- Transfer the 100 mg of grind sample to 1.5 ml tube
- Add **450µl of RPL1 Buffer** and **4.5µl of ß-mercaptoethanol** and vortex vigorously.
- Transfer the lysate (400µl) mix to Filter column and centrifuge for 1 min. at 13,000 rpm.
- Discard the Filter column.

3. Binding

- Add **1/2 volume (200µl) of Ethanol** to filterate and mix by pipetting 5 times.
- Connect Spin Column to 2.0 ml Collection tube.
- Apply the mixture to the Spin Column and centrifuge for 30 sec. at 13,000 rpm.
- Discard the flow-through and re-connect with the Spin Column.

(Optional) DNA residue degradation

Add 100µl of DNase I solution (2U/µl) in the center of Spin Column matrix and incubate for 10 min. at R/T.

4. Wash

- Add **700µl of RPW1 Buffer** to the Spin Column and centrifuge for 30 sec. at 13,000 rpm.
- Discard the flow-through and re-connect with the Spin Column.
- Add **500µl of RPW2 Buffer** (ethanol added) in the center of Spin Column matrix and centrifuge for 30 sec. at 13,000 rpm.
- Add **500µl of RPW2 Buffer** (ethanol added) in the center of Spin Column matrix and centrifuge for 2 min. at 13,000 rpm.
- Discard the flow-through and re-connect the Spin Column and centrifuge for 1 min. at 13,000 rpm.

5. Elution

- Connect the Spin Column and new 1.5 ml tube.
- Add **50µl of DNase/RNase-Free Water** and incubate at R/T for 3 min.
- Centrifuge for 1 min. at 13,000 rpm.
- Discard the Spin Column and eluted purified RNA for use next step.

6. RNA Storage

- The purified RNA sample may be stored at −20°C for a few days. It is recommended that samples be placed at −70°C for long term storage.

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TROUBLESHOOTING

Problem	Possible cause and solution
RNA is degraded / no RNA obtained	RNase contamination • Create an RNase free working environment. Wear gloves during all steps of the procedure. Change gloves frequently. Use of sterile, disposable polypropylene tubes is recommended. Keep tubes closed whenever possible during the preparation. Glassware should be oven-baked for at least 2 hours at 250°C before use.
Poor RNA quality or yield	Insufficient homogenization of plant material. • To disrupt the cell wall, it is important to homogenize the sample thoroughly until it is ground to fine powder. Excess sample used during lysate preparation. • Reduce the amount of starting material. Do not use more than 100mg of plant tissue per column. Ethanol was not added to the lysate. • Ensure ethanol was added to the lysate before applying the sample to the purification column. Ethanol was not mixed properly with the lysate. • After the addition of ethanol to the lysate, mix the sample by vortexing or pipetting. Ethanol was not added to RPW2 Buffers. • Ensure ethanol was added to RPW2 Buffer before use. Sample may be old or degraded • If possible use young leaves or tissues.
Low $_{A260 / A230}$ ratio	Carry-over of guanidinium thiocyanate • Carefully load the lysate to the RNA Spin Column and try to avoid a contamination of the upper part of the column and the column lid. • Make sure that a sufficient amount/concentration of RNA is used for quantification so that the A230 value is significantly higher than the background level.
Clogged Spin Column	Clarified supernatant contaminated with cell debris. • Make sure not to transfer any pelleted precipitate from the lysate clarifying centrifugation step onto spin column. To low temperature during centrifugation. • At low temperatures nucleic acids can precipitate and clog the column. Maintain 20-25°C temperature during centrifugation steps.
DNA contamination	Too much cell material used • Reduce quantity of cells or tissue used. The amount of gDNA co-purified with the sample RNA varies with the plant species used and can be substantial. To completely remove gDNA digest the RNA preparation with DNase I (not provided).
Suboptimal performance of RNA in downstream experiments	Carry-over of ethanol or salt • Do not let the flow-through touch the column outlet after the second RPW2 Buffer wash. Be sure to centrifuge at the corresponding speed for the respective time in order to remove ethanolic RPW2 Buffer completely. • Check if RPW2 Buffer has been equilibrated to room temperature before use. Washing at lower temperatures lowers efficiency of salt removal by RPW2 Buffer. Store isolated RNA properly • Eluted RNA should always be kept on ice for optimal stability since trace contaminations of omnipresent RNases (general lab ware, fingerprints, dust) will degrade the isolated RNA. For short term storage freeze at -20°C, for long term storage freeze at -70°C.

TOTAL RNA YIELDS FROM 50MG OF FRESH TISSUE

Plant	Tissue	Yield (µg)
Tomato	leaf	50-53
Rice	leaf	20-25
Soy	seeds	5-7
Tomato	leaf	50-53
Corn	leaf seeds	25-30 6-7
Nicotiana tabacum	leaf	17-21
Dandelion	leaf	30-33
Spinach	leaf	25-30
Arabidopsis thaliana	leaf	17-20
Sugar-cane	leaf	4-5
Sunflower	stalk seeds	10-11 10-12
Onion	leaf	10-11
Lettuce	leaf	10-11
Cucumber	fruit	40-45
Lemon	leaf	5-7
Wheat	leaf	60-65
Rape	leaf roots seeds	25-30 20-22 40-42
Lucerne	leaf	35-40
Sugar-beet	roots	6-7

SYMBOL GLOSSARY

REF	Catalogue number
LOT	Batch code
	Temperature limit
	Use-by date
	Manufacturer
RUO	Research use only
	Instructions for use

ORDERING INFORMATION

Product	Cat No.	Package
WizPrep™ Plant RNA Mini Kit	W72650-50	50 prep
	W72650-150	150 Prep

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