Plant RNA extraction Kit

(50 rxns)

Zellbio GmbH (Germany)

CAT No. ZX-22104-50

www.zellx.de

For use with real-time RT-PCR, Northern Blotting, cDNA library construction

!!! Caution: This product is for Research Use Only. Not for *in-vitro* Diagnostics !!!

Table of Contents

Please read this insert completely prior to using the product.

Background

ZellX® plant RNA extraction Kit has been designed for the quick purification of total RNA from a variety of plant tissues without phenol/chloroform treatment. It works based on the ability of RNA to bind to silica membrane in the presence of high concentrations of chaotropic salts.

The plant RNA molecules bind to the silica-based membrane, and impurities such as proteins and nucleases are removed by thorough washing with Wash Buffer. The RNA is then eluted in sterile, RNase-free water. The isolated RNA is ready to use and should be stored at - 70 °C.

Intended use

The purified plant RNA is suitable for use in real-time RT-PCR, Northern Blotting, cDNA library construction, etc.

Materials supplied in the Kit

Component	*Quantity*
Minispin-column	50
Filter Columns	50
Collection tubes	100
RNase-free Water	5 mL
Lysis Buffer 1	30 mL
Lysis Buffer 2	30 mL
Wash Buffer 1	40 mL
Wash Buffer 2	20 mL
DNase I Solution	1.5 mL

Storage instruction

All reagents should be kept at room temperature (RT) except the vial of DNase I which must be stored at 4 °C upon receipt.

Materials required but not supplied

Precision pipettes and disposable filter pipette tips (Nuclease-free)

Vortex and micro-centrifuge (10000 × g)

Sterile 1.5 mL micro-centrifuge tubes (Nuclease-free)

Ethanol 96%-100% (we recommend BioUltra for molecular biology, from Sigma-Aldrich Cat. No. 51976)

β-mercaptoethanol (β-ME)

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

β-ME is toxic and must be used under the fume hood and with appropriate protective clothes.

General remarks

- The instruction must be strictly followed.
- Pipette tips should not be used more than once to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

Reagent preparation

i. **Lysis Buffer 1 and Lysis Buffer 2 Working Solution:** Add 600 µL of β-mercaptoethanol (β-ME) [Not included] to each bottle of Lysis Buffers (10 µL of β-ME per mL of lysis buffer) prior to initial use.

ii. **Wash Buffer 2:** Add the appropriate amount (according to bottle label) of molecular biology grade ethanol (96%-100%) [Not provided] to Wash Buffer 2 prior to initial use.

Assay Procedure

1. Cut and weigh the plant samples (up to 100 mg) and immediately put them inside a mortar with liquid nitrogen.
2. Grind the sample under liquid nitrogen to a fine powder, and transfer to a 1.5 mL microcentrifuge tube (not provided).
3. Add 500 µL of Lysis Buffer 1 Working Solution (or Lysis Buffer 2 Working Solution for f plant species with high polysaccharide content) and mix by vigorously vortexing.
4. Incubate at room temperature for 5 minutes.

5. Place a Filter-column in a 2 mL Collection tube and transfer the sample mixture into the Filter-column. Centrifuge at maximal speed for 1 minute.
6. Transfer the clarified filtrate lysate in a new 1.5 mL microcentrifuge tube (not provided) and add equal valume of 70% ethanol and mix vigorously by vortexing.
7. Place the Minispin-column in a 2 mL collection tube and transfer all the sample mixture to the column. Centrifuge at full speed for 1.5 minutes and discard the flow-through.
8. Add 250 µL of Wash Buffer 1 and centrifuge at full speed for 1.5 minutes. Discard the flow-through.
9. Place the Minispin-column in a collection tube and add 60 µL RNase-free DNase I solution (0.5 U/µL) to the centre of the column matrix.
10. Incubate at room temperature for 15 minutes.
11. Add 500 µL of Wash Buffer 1 and centrifuge at full speed for 30 seconds. Discard the flow-through.
12. Add 750 µL of Wash Buffer 2 and centrifuge at full speed for 1 minute. Discard the flow-through
13. Repeat the washing step (step 12) once more using 750 µL of Wash Buffer 2.
14. Centrifuge at full speed for an additional 3 minutes to dry the Minispin-column.
15. Place the Minispin-column into a new, labelled 1.5 microcentrifuge tube (not provided) and pipet 50 µL of RNase-free Water directly onto the membrane (The tip should not touch the membrane). Close the cap and incubate for 1 minute at room temperature.
16. Centrifuge at maximum speed for 1 minute to elute RNA and store it at -70°C.