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PLANT TOTAL RNA MINI KIT RE-007 & RE-008

Description: The Plant Total RNA Purification Mini Kit is designed for purification of total RNA from plant tissues and cells using the modified salt precipitation procedure and RNase inhibitors without the use of hazardous solvents such as phenol. Plant RNA is quickly and efficiently isolated and is immediately available for downstream applications, including RT-PCR, Northern blotting, primer extension and cDNA library construction.

Kit Contents:

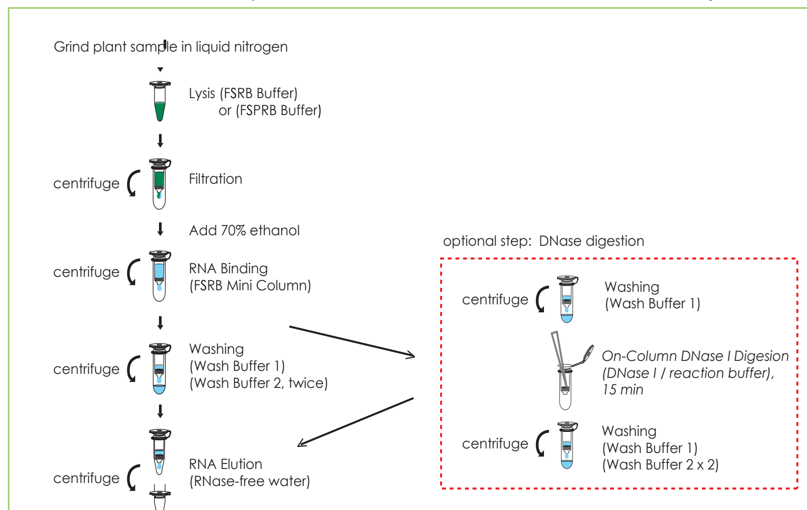
Cat. No:	RE-007s (4 preps-sample)	RE-007 (50 preps)	RE-008 (100 preps)
FSRB Buffer	3 ml	30 ml	60 ml
FSPRB Buffer	3 ml	30 ml	60 ml
Wash Buffer 1	3 ml	30 ml	60 ml
Wash Buffer 2(concentrate)	1.5 ml	20 ml	35 ml
RNase-free Water	0.5 ml	6 ml	6 ml
Filter Column	4 pcs	50 pcs	100 pcs
FSRB Mini Column	4 pcs	50 pcs	100 pcs
Collection Tube	8 pcs	100 pcs	200 pcs
Elution Tube	4 pcs	50 pcs	100 pcs
User Manual	1	1	1
Preparation of Wash Buffer by adding ethanol (96 ~ 100%)			
Ethanol volume for Wash Buffer[a]	6 ml	80 ml	140 ml

Specification:

Principle: mini spin column (silica matrix)
Sample size: up to 100 mg plant tissue or 1 x10^7 plant
cells Operation time: 30 ~ 60 minutes
Binding capacity: up to 100 µg total RNA/ column
Expected yield: 5 ~30 µg of total RNA from 100 mg of young leave
Column applicability: centrifugation and vaccum
Minimum elution volume: 30 µl

Important Notes:

1. Make sure everything is RNase-free when handling RNA.
2. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
3. We suggest to use (ß-ME), or DTT to better to increase RNA yield. Pipet a required volume of FSRB Buffer or FSPRB Buffer to another RNase-free container and add 10 µl ß-mercaptoethanol (ß-ME) or 10µl of Dithiothreitol per 1ml FSRB Buffer or FSPRB Buffer before use.
4. **Caution: ß-mercaptoethanol is hazardous to human health. perform the procedures involving FSRB Buffer or FSPRB Buffer in a chemical fume hood.**
5. Add required volume of RNase-free ethanol (96~100%) to Wash Buffer 2 when first use.
6. All centrifuge steps are done at full speed (~18,000 x g) in a microcentrifuge.
7. Dilute RNase-free DNase 1 in reaction buffer (1M NaCl, 10 mM MnCl2, 20 mM Tris-HCl, pH 7.0 at 25°C) to final conc. = 0.5 U/µl.



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General Protocol:

Please Read Important Notes Before Starting Following Steps.

1. Grind up to 100 mg plant sample under liquid nitrogen to a fine powder and transfer to a new microcentrifuge tube (not provided). **-- Note: Do not use plant sample more than 100 mg, it will lower the total RNA yield.**
2. Add 500 µl of FSRB Buffer (5ul B-ME or 5ul of DTT added) to the sample powder and vortex vigorously. Incubate at room temp.for 5' Use FSPRB Buffer (B-ME or DTT added) if plant sample contains sticky secondary metabolites such as maize with milky endosperm or mycelia of filamentous fungi.
   -- Note: In order to release all the RNA from sample, it is required to disrupt the sample completely by using a suitable disruptor equipment.
3. Place a Filter Column to a Collection Tube and transfer the sample mixture to the Filter Column. Centrifuge at full speed (~ 18,000 x g) for 1 min.
4. Transfer the clarified supernatant from the Collection Tube to a new microcentrifuge tube (not provided), and adjust the volume of the supernatant. **-- Note: Avoid to pipette any debris and pellet when transfering the supernatant.**
5. Add 1 volume of 70 % RNase-free ethanol and mix well by vortexing.
6. Place a FSRB Mini Column to a Collection Tube and transfer the ethanol added sample mixture (including any precipitate) to the FSRB Mini Column. Centrifuge at full speed for 1 min, discard the flow-through and return the FSRB Mini Column back to the Collection Tube.
7. Repeat step 6 for the rest of the sample mixture.
8. (Optional): To eliminate genomic DNA contamination, follow the steps from 8a. Otherwise, proceed to step 9 directly.

8a. Add 250 µl of Wash Buffer 1 to the FSRB Mini Column, centrifuge at full speed for1 min. Discard the flow-through and return the FSRB Mini Column back to the Collection Tube.
8b. Add 60 µl of RNase-free DNase 1 solution (0.5U/ul, not provided) to the membrane center of FSRB Mini Column. Place the column on the benchtop for 15 min.
8c. Add 250 µl of Wash Buffer 1 to the FSRB Mini Column, centrifuge at ful speed for 1 min. Discard the flow-through and return the FSRB Mini Column back to the Collection Tube.
8d. After DNase 1 treatment, proceed to step 10.

9. Add 500 µl of Wash Buffer 1 to the FSRB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the FSRB Mini Column back to the Collection Tube.
10. Add 750 µl of Wash Buffer 2 to the FSRB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the FSRB Mini Column back to the Collection Tube.
    - Note: Make sure that ethanol has been added into Wash Buffer 2 when first use.
11. Repeat step 10 for one more washing.
12. Centrifuge the FSRB Mini Column at full speed for an additional 3 min to dry the FSRB Mini Column. **-- Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.**
13. Place the FSRB Mini Column to a Elution Tube (provided, 1.5 ml microcentrifuge tube).
14. Add 30 ~ 50 µl of RNase-free ddH2O to the membrane center of the FSRB Mini Column. Stand the FSRB Mini Column for 1 min. **-- Important Step! For effective elution, make sure that RNase-free ddH2O is dispensed on the membrane center and is absorbed completely.**
    --Important : Do not elute the RNA using RNase-free water less than suggested volume (< 30 µl). It will lower the final yield.
15. Centrifuge the FSRB Mini Column at full speed for 1 min to elute RNA.
16. Store RNA at -70C.

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