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Spin HiPure Plant RNA Mini Kit(Polysaccharide & Polyphenol-rich)

Cat No.: EXR0227

Size: 50T

Storage: At RT for 12 months, RNase-free DNase should be stored at 4℃.

Product Description

This kit adopts unique lysis system, need not to use toxic reagent such as beta-mercaptoethanol, phenol, trichloromethane, suitable for polysaccharide & polyphenol-rich plant tissue or fungus sample especially. It is easy to operate and RNA extracted from this kit is high purity, less protein, less genomic DNA and less impurities. It can be directly used in downstream experiments such as RT-PCR, Northern blot, Real Time PCR and construction of cDNA library.

Component of Product

Component	Amount	Note
Column Balance Solution	30ml	
Lysis Buffer	40ml	Prepare before using, it can be stored at 4℃ for 1 month at most after adding beta-mercaptoethanol
Washing Buffer 1 (WB1)	40ml	
Washing Buffer 2 (WB2)	12ml	Add absolute ethanol according to the bottle label before using.
RNase-free DNase (2000 U)	1(bottle)	Stored at 4℃
Membrane Reaction Solution	4ml	
RNase-free ddH_2O (tube)	1ml	
RNase-free ddH_2O (bottle)	15ml	
RNase-free Injector	1(pcs)	
Filter Column	50pcs	
Adsorption Column	50pcs	Sealed in dry environment
2ml Collection Tube	2*50pcs	
1.5ml RNase-free Centrifuge Tube	50pcs	

Experiment Preparation

Reagents Required but Not Provided: beta-mercaptoethanol

1. Preparation of RNase-free DNase mother liquid: extract 550ul RNase-free ddH_2O by using 1ml RNase-free injector, add it in glass bottle with RNase-free DNase (2000U) powder, mix gently, store it at -20℃ after subpackage(it can be stored for 9 months).
   Note: RNase-free DNase can be stored for 6 weeks once its taken -20℃ to 4℃. Do not freeze it

again.

2. Precipitation maybe occure in lysis buffer, heat and dissolve at 60℃, use after recovering to room temperature. Make lysis buffer as the ratio of 1:20(for exapmle: add 50ul beta-mercaptoethanol to 1ml lysis buffer), which can be stored at 4℃ for 1 month at most.

3. Add absolute ethanol in washing buffer 2 (WB2) according to the bottle label before using, and mark it.

Protocol

1. Column Balance: add 500ul column balance solution to adsorption column, centrifuge at 12000rpm for 2 min, remove the waste solution in the collection tube
2. Add 500ul lysis buffer to 1.5ml RNase-free centrifuge tube(check if beta-mercaptoethanol has been added). Add the sample powder(50-100mg) after grinding with liquid nitrogen to 1.5ml RNase-free centrifuge tube which contains 500ul lysis buffer, vortex violently and mix thoroughly until there is no significant precipitation in lysis buffer.

   Note: Use 100mg sample if extracted RNA is less than 10ug; add 700ul lysis buffer if the leaf sample is starch-rich.
3. Centrifuge at 12000rpm for 2min.
4. Put filter column into collection tube, then transfer all reagents from step 2 to the filter column, centrifuge at 12000rpm for 5min, transfer filtrate from collection tube to a new 1.5ml RNase-free centrifuge tube carefully. Try to avoid touching the precipitate of cell debris in collection tube.
5. Add 40% volume of the filtrate absolute ethanol, mix thoroughly(precipitate may appear), transfer the solution and precipitate to adsorption column(adsorption column is placed in collection tube), centrifuge at 12000rpm for 15 seconds, remove the waste solution in the collection tube, put adsorption column into collection tube.
6. Add 350ul washing buffer 1(WB 1) to adsorption column, centrifuge at 12000rpm for 1 min, remove the waste solution in the collection tube, put adsorption column into collection tube.
7. Preparation of RNase-free DNase working solution: add 10ul RNase-free DNase mother liquid into a new RNase-free centrifuge tube, add 70ul membrane reaction solution, mix it gently.
8. Add 80ul RNase-free DNase working solution to the middle of the adsorption column, place it at room temperature for 15 min.
9. Add 350ul washing buffer 1(WB 1) to adsorption column, centrifuge at 12000rpm for 1 min, remove the waste solution in the collection tube, put adsorption column into collection tube.
10. Add 500ul washing buffer 2(WB 2) to adsorption column (please check if absolute ethanol has been added), store at room temperature for 2 min, centrifuge at 12000rpm for 1 min, remove the waste solution in the collection tube, put adsorption column into collection tube.
11. Operate the step 10 again.
12. Put adsorption column into collection tube, centrifuge at 12000rpm for 3min, this step is important, the residual ethanol(ingredient in washing buffer 2)will affect the RNA.
13. Put adsorption column into a new 1.5ml RNase-free centrifuge tube, store at room temperature for 2 min, add 50-100ul RNase-free ddH_2O, store at room temperature for 1 min, centrifuge at 12000 rpm for 1 min to get RNA solution. RNA solution should be used immediately or stored at -80℃ after subpackage.

Note

1. This product is intended for scientific research use only. Do not use it for medical, clinical

diagnosis, treatment, food and cosmetic. Do not store it in ordinary residential area.

2. For your safety and health, please wear the experiment clothes, mask and disposable gloves.