Biospin Polysaccharide Polyphenol Plant Total RNA Extraction Kit

BIOER

Kit Components

Cat#	BSC65S1
Components	**50Tests**
Lysis AG	25mL
Lysis BG	25mL
PLANTaid	2.5ml
PG Buffer	30mL
Wash Buffer	18mL(add 54mL ethanol before use)
RElution Buffer	10mL
Spin column	50
Handbook	1copy

Storage and transportation

- The kit has demonstrated stability of 18 months when stored at room temperature.
- The kit can be transported at room temperature.

Introduction

The kit is a ready-to-use reagent for the isolation of total RNA from plant tissue with polysaccharide and polyphenol. Add Lysis to the processed sample and transfer the mixture to Spin column, and then total RNA can be easily isolated through several washing and eluting steps.

The kit provides a very simple, fast and economical technique to isolate high quality RNA, and can go high-throughput. The pure RNA can be applied extensively in Northern blot, blotting hybridization, poly(A)+ selection、in vitro translation、RNase protect assay, RT-PCR/Real time RT-PCR analysis、construction cDNA library etc.

Apparatus and materials to be prepared by the user

* Sterile 1.5mL microcentrifuge tubes
* Microcentrifuge capable of 14,000rpm
* β- mercaptoethanol
* Liquid nitrogen
* 10μL /100μL /1000μL tips
* Absolute ethanol
* Vortex mixer
* Mortar

Important note

- Add the ethanol (as the volume marked on bottle label) to the wash buffer and mix them well.
- When the room temperature is too low, Lysis AG and Lysis BG will crystallization precipitation, please dissolve in 50℃ degrees water bath before used.

Procedure

1. Lysis selection:

   There are two kinds of lysis buffer and a bottle of PLANTaid in the kit. For most plant samples, Lysis AG can be used for extraction. Samples for poor extraction of AG Lysis, please use Lysis

BG (e.g. Siraitia grosvenorii leaves) or Lysis BG+ PLANTaid (e.g. cotton wool fiber). Reagent preparation：.

a. Lysis AG: Add β - mercaptoethanol to Lysis AG until the final concentration is 2%, i.e. adding 20 μL β - mercaptoethanol into 980 μL Lysis AG.
b. Lysis BG: Add β - mercaptoethanol to Lysis BG until the final concentration is 2%, i.e. adding 20 μL β - mercaptoethanol into 980 μL Lysis BG.
c. Lysis BG+ PLANTaid: Add β - mercaptoethanol to Lysis BG until the final concentration is 2%, and add PLANTaid to the mix until the final concentration is 9%, i.e. adding 20 μL β - mercaptoethanol and 100μL PLANTaid into 980 μL Lysis BG. Mix well.

PS: The Lysis is best used at once, it can be stored in 4 ℃ in a month.

2. Sample pretreatment：
   a. Grinding with liquid nitrogen: The amount of plant tissue is grinded into a fine powder with liquid nitrogen. Weigh 50-100mg powder into the 1.5mL centrifuge tube with 500μL Lysis or 550μL Lysis (BG+ PLANTaid), immediately oscillate vigorously to no obvious lumps.
   b. Grinding with grinder: Firstly, add 500 μL Lysis or 550 μL (BG+PLANTaid) Lysis to the grinding tube, then add 50-100 mg of plant tissue to the grinding tube, cover the tube cap tightly, and place the grinding tube into the grinder. , 60HZ grinding for 90sec. (You can choose the oscillation amplitude and time according to different grinder models)
3. The lysate is centrifuged for 5 minutes at 12,000rpm, carefully transfer all lysate supernatant into a new 1.5mL centrifuge tube.
   Note：If you want to get more RNA, you can try to suck more lysate supernatant, but don't suck precipitation.
4. More precise estimates of lysate supernatant volume, adding absolute ethanol 0.5 times volume. This may appear precipitation, but does not affect the extraction process. Please immediate mix, but does not centrifuge.
5. Transfer all the mixture into a Spin column and centrifuge for 1 minute at 12,000rpm. Discard the flow-through.
   Note：Before the next step,the mixture must pass through the Spin column.
6. Add 600μL PG Buffer into the Spin column and stand at room temperature for 30 seconds. Then centrifuge for 30 seconds at 12,000rpm. Discard the flow-through.
7. Add 500μL Wash Buffer into the Spin column, centrifuge for 30 seconds at 12 ·000rpm. Discard the flow-through. Add 250μL Wash Buffer into the Spin column, repeat step 9.
8. Then centrifuge for an additional 1 minute at 12,000rpm. Try to remove Wash Buffer, so as to prevent the residual ethanol in Wash Buffer inhibiting of downstream reaction.
9. Transfer the Spin column to a RNase-free 1.5mL centrifuge tube. Add 50-100μL RElution Buffer (or RNase-free water pH>7.0) to the central of the membrane; Incubate at the room temperature for 2 minute and then centrifuge for 1 minute at 12,000rpm. Then the buffer in the centrifuge tube contains the total RNA.

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10. If need to increase the RNA concentration ,Reload the elute into the center of the Spin column, Incubate at the room temperature for 2 minute and then centrifuge for 1 minute at 12,000rpm. Then the buffer in the centrifuge tube contains the total RNA.

Troubleshooting

1. We ensure the stability of the kit should be 18 months when stored at room temperature.
2. When the room temperature is too low, Lysis AG and Lysis BG will crystallization precipitation, please dissolve in 50℃ degrees water bath before used.
3. For the first using, please add absolute ethanol to the Wash Buffer and cap the bottle and mix evenly.
4. Please wear gloves and clean all tips and Eppendorf tubes with DEPC-ddH_2O in order to avoid RNase contaminant. If conditions permit, please operate in ultra-clean cabinet.
5. If there are residue liquid left on the tube after transferring the supernatant to the Spin column and 1min centrifuging, it means the excessive sample or incompletely lysis so as to block the membrane. Here is the outlet: ①Reduce sample volume. ②If block appears use tip to smash the surface of membrane and then centrifuge again. Usually, it is recommended to discard the sample.
6. Please ensure to add 50μL-100μL elution buffer in the center of membrane. If the volume of elution buffer is lower than 50μL, it is hardly fully soak the membrane.
7. The total RNA should be stored at 4℃ or in the ice bath in order to be used in the downstream experiment, e.g. RT-PCR; or be stored at -80℃. It is suggest avoiding long time storage because RNA is extremely easy to be degraded even with any methods. It is suggested that to store RNA sample in nitrogen or stock buffer in order to ensure purity of RNA.