CHANGYU Plus Complex Plant RNA Mini Kit

Cat#：CYRN53

❖ Kit Contents and Storage

Kit Contents	Storage	50 Preps (CYRN53)
Lysis Buffer CLB	RT	50 ml
Lysis Buffer RLT Plus	RT	25 ml
Buffer RW1	RT	40 ml
Wash Buffer RW	RT	10 ml *Add indicated ethanol before first use*
RNase-free H_2O	RT	10 ml
Genomic DNA Elimination Columns	RT	50
RNA Bind Columns	RT	50

All reagents, when stored at the indicated temperature, are stable for 9 months.

Storage Note:

1. Inappropriate storage at low temperature (4 °C or -20 °C) will lead to precipitation, which will affect the reagents effect. Therefore, transportation and storage should be at room temperature (15 °C -25 ° C).

2. The solution should be promptly covered tightly after use to avoid exposing the reagents to the air for a long time.

❖ Description

The innovative CHANGYU plus plant system is ideal for rapid purification of total RNA from plant (including complex plant). The unique genomic DNA elimination columns effectively remove genomic DNA from RNA samples. The whole process is phenol/chloroform-free. The unique lysis buffer immediately lyses biological samples and inactivates RNase and DNase. The lysate containing RNA/DNA is then passed through a genomic DNA elimination column, where genomic DNA binds to the column membrane, and RNA is eluted from DNA elimination column. Ethanol is added to the flow-through to provide appropriate binding conditions for RNA, and RNA selectively binds to the silica-membrane of the RNA column in the high-salt buffer. RNA is purified through a series of wash-spin steps to remove protein followed by elution of RNA from silica-membrane with RNase-free H2O.

Product features

1. Security: no phenol and chloroform, no ethanol precipitation and other steps.

2. Simplicity: Single sample operation is generally able to be completed in 30 minutes.

3. Originality: Exclusively developed genomic DNA removal column technology to remove gDNA residues effectively. The obtained RNA generally does not require DNase digestion, and can be used for reverse transcription, real time PCR and other experiments directly.

4. Versatility: The kit can be used to extract, including complex plant such as Chinese herbal medicines: Dendrobium, Salvia, Saussurea, Ginseng; complex starch seeds such as rice, wheat and corn seeds; complex fruits such as grape, blueberry, strawberry and watermelon,

5. Purity: Multiple column washing ensures high purity. Typical OD260/OD280 ratios range from 2.0 to 2.2 with no DNA contamination.

PRECAUTIONS

1. All centrifugation steps can be finished at room temperature.

2. β-mercaptoethanol and ethanol are needed which are not included in the kit.

3. Samples should never exceed the capacity of the genomic clearance column, and capacity of RNA binding column.

4. About the trace of gDNA residues:

A. Generally, DNase digestion is not required with CHANGYU plus Kits since silica membrane technology efficiently removes most of the DNA without DNase treatment. However, further DNA removal may be necessary for certain RNA applications that are sensitive to traces of DNA (e.g., TaqMan RT-PCR analysis with a low-abundance target). In these cases, residual DNA can be removed by optional on column DNase digestion using the RNase-Free DNase I. The DNase is efficiently removed in subsequent wash steps. Alternatively, residual DNA can be removed by a DNase digestion after RNA purification. The DNase digestion can then be cleaned up, if desired, using “Protocol: RNA Cleanup”. (send to you on inquiry).

B. If the rigorous analysis such as RT-qPCR will be carried out and the trace of gDNA residues are not allowed, then the primers across boundary of Intron and Exon are recommended. Those primers can not be used to amplify genomic DNA.

PROTOCOLS (Please read the precautions before the experiment):

IMPORTANT: Please add indicated ethanol in the **wash buffer RW** before first use, mix well, and mark the bottle with a check.

Take 1ml of **Lysis buffer CLB** into the centrifuge tube (if CLB is precipitated, place on a 65 °C water bath to redissolve precipitation) and add 5% β-mercaptoethanol (1 ml CLB plus/50μl β-mercaptoethanol) to make working Buffer CLB. You can make more working Buffer CLB by scaling up proportionately if multiple samples are prepared.

1. Direct grinding method (recommended for lab without liquid nitrogen or plant samples which were easy to grind):

a. Put 100-200mg fresh plant tissue or preserved frozen samples which had been cut into small pieces quickly into a mortar, adding 1ml CLB (β-mercaptoethanol has been added) to fully grind the samples into a homogenate at room temperature. Please note: The grinding of samples after adding CLB buffer should be as quickly as possible to inhibit RNase activity.

Important: β-mercaptoethanol is a key component of the buffer CLB. So its final concentration can be increased to 10%-20% if necessary.

For particularly complex plants, trying to add PVP40 to the buffer CLB to a final concentration of 2%.

b. Transfer the lysate into a centrifuge tube and immediately shake vigorously for 15 seconds. Placing the lysate in a 65 °C water bath for 5-10 mins with an occasional 1–2 times vortex to help lysis. Centrifuging at 13,000 rpm for 10 minutes to precipitate the cell-debris that have not been lysed.

c. Take the supernatant to a new centrifuge tube (If there were floats on the surface of the supernatant, just pipette the supernatant below). Pipette more supernatant to the column without exceeding the capacity of the genomic DNA elimination column may increase yield. **Add half volume anhydrous ethanol of the supernatant (0.5 volume) and mix immediately by pipetting. Do not centrifuge.** Please note: when the absolute ethanol added, some substance may precipitate, but this does not affect the extraction process.

d. Immediately follow step 3 of the protocol.

2. **Liquid nitrogen grinding method (recommended for complex and difficult to extract or easy to degrade samples)**:

a. Grind fresh or -80°C frozen material to powder in a mortar with liquid nitrogen as quickly as possible.

b. Transfer 100-200mg grinded material powder to the 65°C preheated lysate buffer CLB (with β-mercaptoethanol) in centrifuge tube. Vortex vigorously for 30-60 seconds or pipette well to get the completed homogenates.

c. Put the homogenates into the 65°C water bath for about 5-10 mins with the occasional 1–2 times vortex to help lysis.

d. Centrifuge at 13,000 rpm for 10 minutes to precipitate the cell-debris that have not been lysed.

e. Take the supernatant to a new centrifuge tube (If there were floats on the surface of the supernatant, just pipette the supernatant below). Pipette more supernatant to the column without exceeding the capacity of the genomic DNA elimination column may increase yield. **Add half volume anhydrous ethanol of the supernatant (0.5 volume) and mix immediately by pipetting gently. Do not centrifuge. Please note**: when the absolute ethanol added, some substance may precipitate, but this

does not affect the extraction process.

f. Immediately follow step 3 of the protocol.

3. Transfer up to 720 µl mixture to the new Genomic DNA Elimination Column placed in a 2 ml collection tube (provided). Centrifuge at 13,000 rpm for 2 minutes, and discard the flow-through. Repeat this step if the sample volume exceeds 720 µl. Make sure the liquid is completely filtered after centrifugation and there is no residue liquid on the membrane.

4. Take the Genomic DNA Elimination column to a clean 2 ml centrifuge tube. Add 500 µl Lysis Buffer RLT Plus. Centrifuge at 13,000 rpm for 30 seconds, and save the flow-through (RNA is in the flow-through). Add half volume anhydrous ethanol of the flow-through (0.5 volume). (usually 450–500 µl, adjust the ethanol volume accordingly if some lysis buffer is lost during the above procedure). Precipitation may be formed after the addition of ethanol, but this does not affect the procedure. Pipette gently to mix immediately. **Do not centrifuge**.

5. Transfer up to 720 µl mixture to the new RNA Binding Column placed in a 2 ml collection tube (provided). Centrifuge at 13,000 rpm for 2 min, and discard the flow-through. Repeat this step if the sample volume exceeds 720 µl. Make sure the liquid is completely filtered after centrifugation and there is no residue liquid on the membrane.

6. Add 700 µl Buffer RW1, and incubate at room temperature for 1 minute. Centrifuge at 12,000 rpm for 30 seconds and discard the flow-through.

7. Add 500 µl wash buffer RW (check to see if ethanol has been added!). Centrifuge at 12,000 rpm for 30 seconds and discard the waste. Add 500 µl wash buffer RW and repeat for one time.

8. Place the RNA binding column back into the same collection tube. Centrifuge the empty column at 13,000 rpm for 2 minutes to completely remove ethanol from the column.

9. Place the column in a RNase free centrifuge tube. Add 30–50 µl of RNase free water (Optional: pre-warm the water to 70°C–90°C will increase the RNA yield) to the center of the column membrane. Incubate at room temperature for 1 min, and centrifuge at 12,000 rpm for 1 min to elute the RNA.

10. If the expected RNA yield is >30 µg, repeat step 9 with another 30–50 µl of RNase-free water, or use the eluate from step 9 (if high RNA concentration is required). Reuse the centrifuge tube from step 9. If using the eluate from step 9, the RNA yield will be 15–30% less than that obtained using a second volume of RNase-free water, but the final RNA concentration will be higher.

Note: If using the eluate from step 9, the RNA yield will be 15%–30% less than that obtained using a second volume of RNase-free water, but the final RNA concentration will be higher.

This product is furnished for LABORATORY RESEARCH USE ONLY.
Not for diagnostic or therapeutic use.