V02

Column extraction of plant miRNA kit

Cat: R2230
Package: 50T/100T
Storage: RT, Valid for 1 year.

Product composition:

Kit composition	50T	100T
Lysate	35mL	70mL
Bleach solution	15mL	30mL
RNase-free ddH_2O	2mL	4mL
Adsorption column	100	200
2mL collection tube	100	200
Specification	1	1

Notes:

1. Please add anhydrous ethanol to the bleach solution before use. Please refer to the label on the bottle to add the volume.
2. Bring your own chloroform.

Product description:

This kit uses a unique lysate to replace traditional Trizol, and can rapidly extract miRNA from plant tissues, and the extracted miRNA has high purity and concentration, without other impurities, and can be used for a variety of downstream experiments such as RT-qPCR.

Operation procedure:

1. Add 500μL lysate into 1.5mL centrifuge tube, grind an appropriate amount of fresh plants into fine powder in liquid nitrogen, transfer 50-100mg of fine powder into the centrifuge tube containing lysate, swirl for 15s, and let stand at room temperature for 5min.
2. Add 100μL chloroform to the above cracked liquid, swirl and shake for 30s, and let stand at room temperature for 5min.
3. Centrifuge at 12000rpm for 10min at 4℃.
4. Measure the volume of supernatant liquid and slowly transfer it to a new 1.5mL centrifuge tube, then add anhydrous ethanol with 0.45 times the volume of supernatant liquid (such as 400μL supernatant and 180μL anhydrous ethanol) into it, and swirl for 5s.
5. Add the above mixture to the adsorption column at one time (the adsorption column is placed in the collection tube), centrifuge at 12000rpm for 2min, and collect the filtrate.
6. Measure the filtrate volume, slowly transfer to a new 1.5mL centrifuge tube, then add 0.65 times the filtrate volume of anhydrous ethanol (such as: 500μL filtrate and 325μL anhydrous ethanol) into it, and swirl for 5s.
7. Add the above mixture into a new adsorption column once or twice (the adsorption column is placed in the collection pipe), centrifuge at 12000rpm for 2min, and discard the filtrate.

[Note]

If the filtrate volume does not exceed 500μL, the mixture can be added to the adsorption

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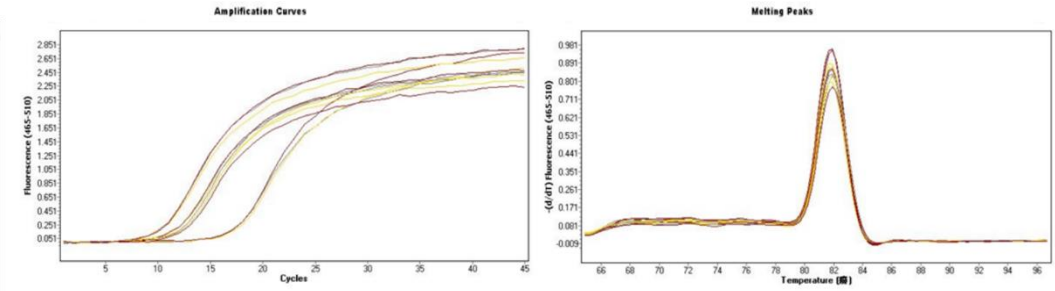
column at one time, otherwise it needs to be added twice.

8. Put the adsorption column back into the collection tube, then add 600μL bleach solution to the adsorption column, centrifuge at 12000rpm for 1min, and discard the filtrate.
9. Repeat 8.
10. Put the adsorption column back into the collection tube, centrifuge at 12000rpm for 2min, discard the collection tube, and open the adsorption column for 2min to remove the residual ethanol.
11. The adsorption column was put into a new 1.5mL centrifuge tube, and 30μL RNase-free ddH_2O was added to the center of the adsorption column and left for 5min at room temperature. miRNA solution was obtained by centrifugation at 12000rpm for 2min at 4℃.

Notes:

1. All relevant utensil consumables should be RNase-free products. Be careful during operation. Wear masks and gloves to avoid contamination of samples with RNA enzymes in the environment.
2. Avoid volatilization, oxidation and pH value changes caused by long-term exposure to the air, and cover the solution tightly in time after use.
3. miRNA was extracted from fresh plant tissues as far as possible.

Example:



The extracted miRNAs can be used to detect specific primers:

The quality of miRNA extracted by this kit was tested, and the samples included plant roots, stems, leaves and other tissues. In order to further test whether the miRNA extracted by this kit could be specifically applied to fluorescent quantitative PCR, U6 universal primers for the leaves of green plants and chlorophylla were designed for verification. Through fluorescence quantitative PCR analysis, it was found that the dissolution curves were relatively neat. It indicates prim-specific amplification. Moreover, the specificity of the melting curve is good and the CT values of different samples are close, indicating that this method can obtain miRNA and can be stably applied to the fluorescence quantitative PCR detection of specific primers.

Related products:

R1600 DEPC Treating water
R1050 5×RNA Loading Buffer
M1010 10×MOPS Buffer
R2200 Column extraction of whole blood miRNA kit
R2220 Column extraction of tissue cell miRNA kit