

Hieff™ Plant Plus RNA Kit

The Hieff™ Plant Plus RNA Kit adopts Hieff™ RNA adsorption Column and a new solution system, which is suitable for efficient extraction of high-purity, high-quality total RNA from plant tissue samples rich in polysaccharides and polyphenols. The operation is simple, and the extraction of total RNA can be completed in 30 minutes. The DNA-Removing Column in the kit can easily adsorb and remove genomic DNA, and Hieff™ RNA Column can efficiently bind RNA. The extracted total RNA is of high purity and can be used in a variety of molecular biology experiments such as RT-PCR, Real-Time PCR, molecular cloning, and RNase protection analysis.

Specifications

Cat.No.	19292ES08 / 19292ES50
Size	5T / 50T

Components

Components No.	Name	19292ES08	19292ES50
		5 T	50 T
19292-A	RNA Adsorption Column P5	5	50
19292-B	2 mL Collection Tube P5	10	100
19292-C	DNA-Removing Column P5	5	50
19292-D	Lysis Buffer(LB Buffer P5*)	2.5 mL	25 mL
19292-E	Helper Buffer B5	600 µL	6 mL
19292-F	Binding Buffer(BD Buffer P5*)	2.4 mL	24 mL
19292-G	Deproteinization Solution PL(PL Buffer P5)	2.5 mL	25 mL
19292-H	Wash Buffer P5*	2.4 mL	24 mL
19292-I	RNase-free H ₂ O	1 mL	10 mL

Storage

Transport and store at room temperature (15-25°C), valid for 2 years.

Notes

1. Avoid volatilization, oxidation, and pH change of the reagent caused by long-term exposure to the air, and cover the solution in time after use.
2. Pay attention to observe whether each solution has precipitation or turbidity (especially in winter when the room temperature is low). You can re-dissolve it in a 37°C warm bath until the solution is clear to avoid affecting the use effect.
3. Binding Buffer/BD Buffer P5*(19292-F) and Deproteinisation Solution/PL Buffer P1(19292-G) contains irritating compounds. Please operate with lab coats and disposable gloves, for your safety.
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5. This product is for research use only.

Pre-experiment preparation

1. Self-provided equipment and reagents: desktop centrifuge, shaker, water bath pot or metal bath, anhydrous ethanol, centrifugal tube, etc.
2. Unless otherwise specified, all centrifugation steps are completed at room temperature, and a traditional desktop centrifuge with a rotating speed of 12,000 rpm is used.
3. Before the first use, add 20 μ L of β -mercaptoethanol to 1 mL of Lysis Buffer(19292-D). It is recommended to prepare and use it immediately. After adding β -mercaptoethanol, store the LB Buffer* in aliquots at 4°C. It is stable for several weeks.
4. Before the first use, add the specified volume of anhydrous ethanol to the Binding Buffer/BD* bottle (taking the 50 T specification as an example, 24 mL of Binding Buffer needs to be added with 30 mL of anhydrous ethanol), mix it thoroughly before use, and mark it. Close the bottle cap tightly after each use to maintain the ethanol content in the Binding Buffer*.
5. Before the first use, add the specified volume of anhydrous ethanol to the Wash Buffer* bottle (taking the 50 T specification as an example, 24 mL of Wash Buffer* needs to be added with 60 mL of anhydrous ethanol), mix it thoroughly before use, and mark it. Close the bottle cap tightly after each use to maintain the ethanol content in the Wash Buffer*.

Instructions

Part I sample pretreatment

- 1.1 According to the number of samples, LB Buffer P5(19292-D) and β -mercaptoethanol (self-prepared) were mixed into **the reaction buffer** at a volume ratio of 500:10. The amount of action buffer required for each sample is 510 μ L. The prepared action buffer was dispensed into 1.5 mL RNase-free centrifuge tubes for later use.
- 1.2 Take the chopped fresh plant tissue and put it into a mortar (need to be dry-baked at 180°C and pre-cooled), and grind it into fine powder with liquid nitrogen.

【Note】 : Liquid nitrogen needs to be added continuously during the grinding process.

- 1.3 Quickly transfer 50 mg of fine powder to an RNase-free centrifuge tube containing containing reaction buffer(Step 1.1 Prepared), immediately vortex for 1 min and leave for 5 min at room temperature.
- 1.4 Add 100 μ L Helper Buffer B5(19292-E).
- 1.5 (Optional step) Centrifuge at 12,000 rpm for 5 min and carefully transfer the supernatant to a new 1.5 mL RNase-free centrifuge tube.

【Note】 : Perform this step only when there are obvious tissue fragments in the lysis mixture.

Part II RNA extraction

- 2.1 Put the DNA- Removing Column(19292-C) into a 2 mL collection tube for later use.
- 2.2 Add the above lysis mixture to the DNA-Removing Column, centrifuge at 12,000 rpm for 5 min, and retain the filtrate.

【Note】 : After centrifugation, if there is still liquid remaining on the filter membrane, increase the centrifugation speed or extend the centrifugation time to 10 min.

2.3 Carefully transfer the supernatant from the filtrate to a new 2 mL RNase-free centrifuge tube (self-prepared), add 1.5 times the volume of the supernatant in Binding Buffer*(19292-F), and mix gently.

【Note】 : Make sure that anhydrous ethanol has been added to Binding Buffer*.

【Note】 : Precipitation is normal. Do not centrifuge and proceed directly to the next step.

2.4 Add all the above mixed solution to the RNA adsorption column, centrifuge at 12,000 rpm for 1 min, and discard the filtrate.

【Note】 : The maximum volume of the mixed solution added each time is 700 μ L, and it can be centrifuged multiple times.

【Note】 : After centrifugation, if there is still liquid remaining on the filter membrane, the centrifugation time can be extended to 5 min.

2.5 Add 500 μ L of Deproteinization solution (PL Buffer P5*,19292-G), centrifuge at 12,000 rpm for 30 s and discard the waste liquid.

2.6 Add 700 μ L anhydrous ethanol (self-prepared), centrifuge at 12,000 rpm for 1 min, and discard the waste liquid.

2.7. Put the RNA adsorption Column(19292-A) back into the collection tube, add 700 μ L of Wash Buffer(19292-H)*, centrifuge at 12,000 rpm for 1 min, and discard the waste liquid.

【Note】 : Make sure that anhydrous ethanol has been added to the rinsing solution W*.

2.8. Repeat step 7.

2.9. Put the RNA Adsorption Column back into the collection tube and centrifuge the empty column at 12,000 rpm for 2 min to remove the residual Wash Buffer(19292-H)*.

2.10. Put the RNA Adsorption Column into a new 1.5 mL RNase-free centrifuge tube, add 50-200 μ L RNase-free H₂O(19292-I) to the center of the Adsorption Column, and leave it at room temperature for 2 min. Then centrifuge at 12,000 rpm for 1 min. Collect the filtrate, which is the RNA solution.

【Note】 : The recovery yield can be increased by the following methods: ① Preheat RNase-free H₂O at 65°C; ② Load the RNA filtrate onto the Column again, leave it at room temperature for 2 min, and then elute.

2.11. The RNA solution can be stored at -80°C for a long time.