FastPure Plant DNA Isolation Mini Kit

DC104-01

Version 9.1

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Introduction

FastPure Plant DNA Isolation kit is specially designed for genomic DNA isolation from fresh and dry plant samples or plant samples with high content of polysaccharides and polyphenolics. The kit uses silica gel membrane purification technology and a new unique solution system, no toxic reagents such as phenol chloroform are needed, and no time-consuming alcohol precipitation is required during the isolation process, so that proteins and other organic compounds in plant cells can be removed maximally. The isolated genomic DNA has high purity and stable quality, and can be used in molecular biological experiments such as PCR, restriction enzyme digestion and hybridization. The amount of DNA isolated varies from different plant tissue.

Package Information

Components	RC401-01 (50 rxn)
RNase A	250 ul
Buffer A1	25 ml
Buffer A2	10 ml
Buffer A3	15 ml
Buffer AW	15 ml
Elution Buffer	15 ml
FastPure gDNA Columns IV	50 each
Collection Tubes 2 ml	50 each

RNase A: Enzymatic hydrolysis of RNA in samples
Buffer A1: Provides a sample cracking environment;
Buffer A2: removal of impurities such as proteins and cell debris;
Buffer A3: Provides the upper column environment;
Buffer AW: Removes salt ions from DNA;
Elution Buffer: Elutes the DNA on the adsorption column;
FastPure gDNA Columns IV: Adsorption of genomic DNA;
Collection Tubes 2 ml: Filtrate collection tube.

Storage

RNase A should be stored at -30℃ ~ -15℃, and be shipped at -20℃ ~ 0℃. Other contents of the FastPure Plant DNA Isolation kit are shipped at room temperature. Upon receipt, store the component at room temperature (15℃ - 25℃).

Application Range

≤ 100 mg fresh plant sample.
≤ 20 mg of dried plant samples.

User–preparation

anhydrous ethanol, 1.5 ml sterilization centrifuge tube, mortar, water bath or metal bath. (Optional: PVP-40, β-Mercaptoethanol)

Notes

◇ While using the kit for the first time, please refer to the label on the bottle, add the corresponding amount of absolute ethanol to Buffer A3 and Buffer AW, mix and use.

◇ If the kit is stored at a low temperature, check whether there is any precipitate in Buffer A1 and Buffer A3 before use. If there is precipitation, it can be left at room temperature for a short time. If necessary, it can be dissolved in the 65℃ water bath until the precipitate is dissolved (Buffer A3 can be heated if it without ethanol, it can't be heated after adding ethanol), and mix it before use.

◇ The amount of adding sample do not exceed the compatibility of the kit, otherwise the sample lysis will be insufficient.

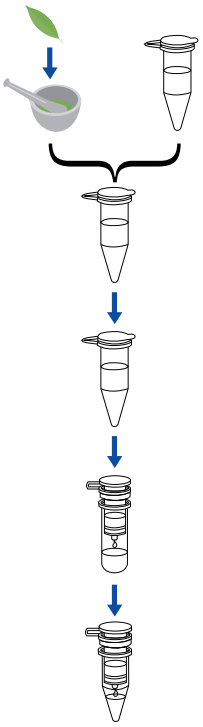
◇ Avoid liquid nitrogen frostbite and centrifuge tube explosion caused by temperature difference; Replenish liquid nitrogen timely during grinding to prevent liquid sample from melting. If not immediately proceed to the next step, please store the grinded sample at -70℃ and avoid repeated freezing and thawing.

◇ It is recommended to perform the operation in the fume hood.

◇ Buffer A3 contains irritating compounds. Wear disposable latex gloves and a mask to protect your skin, eyes and clothing. If you are contaminated with skin or eyes, please rinse with plenty of water or saline, and seek medical advice if necessary.

◇ All operation steps are carried out at room temperature (15℃ - 25℃).

Experimental Principle and Process Summary



◇ Sample grinded in liquid nitrogen : 100 mg fresh plant / 20 mg dry tissue

◇ Cleavage sample tissue: add 400 ul of Buffer A1, 4 ul of RNaseA (10 mg/ml), 65℃ water bath for 10 min

◇ Remove impurities such as protein: add 130 ul of Buffer A2, place in ice for 5 min. 14,000 rpm for 5 - 10 min, take the supernatant

◇ Adjust the column environment: Add 1.5 times the supernatant volume of Buffer A3, mix by pipetting immediately

◇ Genome adsorption: transfer the mixture to the adsorption column FastPure gDNA Columns IV, centrifuge at 12,000 rpm for 30 - 60 sec

◇ Removal of salt ion: Add 600 ul BufferAW, centrifuge at 12,000 mpm for 30 sec (twice)

◇ Removal of ethanol: centrifuge the empty column at 12,000 pm for 2 min

◇ Genome elution: 50 - 100 ul preheated elution Buffer, 2 min at room temperature, and centrifuge at 12,000 rpm 1 min.

Protocol

Sample Preparation

100 mg of fresh plant tissue or 20 mg of dried plant tissue was taken, thoroughly ground into a powder by adding liquid nitrogen, and the powder was transferred to a 1.5 ml centrifuge tube.

▲The sample processing volume should not exceed the compatibility of the kit, otherwise the sample lysis will be insufficient; for samples with high moisture content, such as strawberry, watermelon, etc., the sample size may be increased.

▲If the sample after liquid nitrogen grinding is not immediately subjected to the next step, please store it at -70℃ to avoid repeated freezing and thawing.

DNA Isolation

1. Immediately add 400 ul of Buffer A1 and 4 ul of RNase A (10 mg/ml) to the ground sample powder, vortex and mix thoroughly to help to lysis.
   ▲Optional: 2% PVP-40 can be added to Buffer A1 when the polysaccharide content is particularly high; 0.2% β-mercaptoethanol can be added to Buffer A when the polyphenol content is particularly high. You can also add both at the same time.
2. 65℃ water bath for 10 min. Invert the tube for 2 - 3 times during the water bath process to mix the sample.
3. Add 130 ul Buffer A2 to the mixture, mix thoroughly, place on ice for 5 min, centrifuge at 14,000 rpm (-18,400 × g) for 5 - 10 min, carefully pipet the supernatant to a new 1.5 ml centrifuge tube (Self-provided). Be careful not to pipette the interface material.
4. Add 1.5 times the supernatant volume of Buffer A3 (check whether you have added absolute ethanol before use), such as if the supernatant is 500 ul, add 750 ul of Buffer A3 to it, and mix by pipetting immediately.
   ▲ Adding Buffer A3 may cause flocculation, and you can proceed to the next step after mixing.

5. Transfer the mixture obtained in the previous step (including the precipitate) to FastPure gDNA Columns IV (the adsorption column has been placed in the collection tube) and centrifuge at 12,000 rpm (-13, 400 × g) for 30 - 60 sec, and discard the filtrate.
   ▲The volume of the adsorption column is 700 ul. Please centrifuge for several times.
6. Add 600 ul of Buffer AW (check if anhydrous ethanol has been added before use), centrifuge at 12,000 rpm (-13,400 × g) for 30 sec, and discard the filtrate.
7. Repeat step 6.
8. Place the adsorption column back into the collection tube and centrifuge at 12,000 rpm (-13, 400 × g) for 2 min to remove the rinse solution as much as possible to prevent residual ethanol from inhibiting the downstream reaction.
   ▲ After centrifuged, Open the lid for 2 - 5 min to completely evaporate the residual ethanol.
9. Place the column in a new 1.5 ml centrifuge tube (Self-provided). Add 50 - 100 ul preheated to 65℃ -70℃ Elution Buffer to the center of the adsorption column, stand at room temperature for 3 - 5 min, centrifuge at 12,000 rpm (~13,400 × g) for 1 min.
   ▲ The elution volume should not be less than 50 ul, or the elution efficiency will be decrease below it below 50 ul;
   ▲ Repeat the elution step with the new Elution Buffer, which may increase yield but decrease concentration
   ▲ If you need to get the highest yield, it is recommended to re-add the first eluent to the adsorption column and repeat the elution step.
10. Discard the adsorption column and store the DNA in -20℃.

Troubleshooting and FAQ

FAQ	Reasons	Solutions
Columns was blocked	1. The sample is not fully ground and cracked, and there are many clumps.	Grind thoroughly, mix immediately after adding lysate
	2. The lysate is too viscous	Reduce the initial amount of sample to avoid over-treatment
	3. The centrifugal force is too small	Increase centrifugal force
Low yield of DNA	1. Excessive sample amount and incomplete lysis	Reduce the amount of sample and grind it sufficiently
	2. Inappropriate binding conditions	Estimate the supernatant accurately in Step 4, and accurately add 1.5 times the volume of Buffer A3 to the supernatant
	3. Residual ethanol remain in adsorption column	Ensure that the empty column is centrifuged for 2 minutes and the cap is opened for several minutes to completely volatilize the residual ethanol
	4. No anhydrous ethanol was added to Buffer AW	Adding the corresponding amount of anhydrous ethanol
	5. Insufficient elution	The eluent should be added to the center of the membrane of the column. And increase the volume or times of elution
DNA solution with color or pigment remain on membrane	1. The number of rinses is not enough	After step 7, add 500 µl of anhydrous ethanol and rinse again.
	2. Excessive starting amount of sample	Reduce the starting amount of the sample and avoid excessive
Downstream results are not ideal	1. Ethanol pollution	Make sure to centrifuge the empty column for 2 min, and open the lid is for a few minutes to completely evaporate the residual ethanol
	2. Silicon components fall off	Centrifuge the eluted genomic DNA again at 12,000 rpm for 1 min. Carefully take the supernatant for use

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