FastPure Plant DNA Isolation Mini Kit

DC104

Vazyme

Instruction for Use

Version 23.1

Vazyme

Contents

01/Product Description

This kit is suitable for the rapid extraction of genomic DNA from plant tissues, especially those rich in polysaccharides and polyphenols. The kit is based on silica gel membrane purification technology without time-consuming alcohol precipitation and does not require phenol/chloroform, or any other toxic reagent during the extraction process, which can effectively remove impurities and other organic compounds from plant cell. The obtained genomic DNA with high purity and stable quality can be directly used in PCR, qPCR and enzyme digestion, etc. The amount of genomic DNA extracted from different plant tissues will vary.

02/Components

Components	DC104-01 (50 rxns)
RNase A	250 μl
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
Collection Tubes 2 ml	50

RNase A: Used to remove RNA.

Buffer A1: Provide the lysis enviroment.

Buffer A2: Remove protein, cell debris and other impurities.

Buffer A3: Provide the binding environment.

Buffer AW: Remove residual salt ions of genomic DNA.

Elution Buffer: Elute genomic DNA.

FastPure gDNA Columns IV: Adsorb genomic DNA.

Collection Tubes 2 ml: Collect filtrate.

03/Storage

RNase A should be stored at -30 ~ -15℃ and transported at room temperature.

Other components should be stored at 15 ~ 25℃ and transported at room temperature.

04/Applications

≤100 mg fresh plant samples.

≤20 mg dried plant samples.

05/Self-prepared Materials

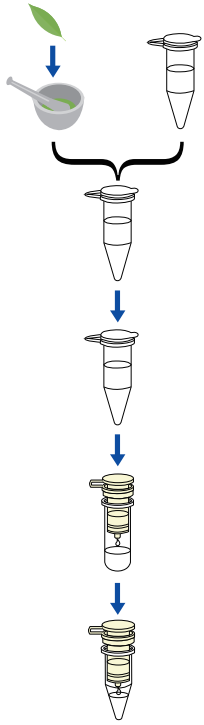
Absolute ethanol, sterilized 1.5 ml centrifuge tube, mortar, and water bath or thermal shaker (Optional: PVP-40, β-Mercaptoethanol).

06/Notes

For research use only. Not for use in diagnostic procedures.

1. Please add the corresponding amount of absolute ethanol to Buffer A3 and Buffer AW according to the label, mark the bottle body and cap and mix well before use.
2. Check whether there is crystal precipitation in Buffer A1 and Buffer A3 before use. If there is crystal precipitation, it can be placed at room temperature. If necessary, place in a 65℃ water bath to dissolve the precipitation (Buffer A3 can be heated without ethanol, but not after ethanol is added), and then mixed thoroughly before use.
3. Do not process more sample than the kit -capacity, as this may result in inadequat sample lysis.
4. Avoid liquid nitrogen frostbite and centrifuge tube explosion caused by temperature difference.Replenish liquid nitrogen during cryogenic grinding to prevent the sample from thawing.Store at -85 ~ -65℃ if the ground sample is not used immediately for the next step.
5. It's recommended to perform experimental operation in fume hood.
6. Buffer A3 contains irritants. Wear latex gloves when handling the buffers and avoid exposure to skin, eyes, or clothing. In case of skin or eye contact, flush with large amounts of water or saline, and seek medical attention if necessary.
7. Perform all steps at room temperature (15 ~ 25℃).

07/Mechanism & Workflow



Liquid Nitrogen Grinding: 100 mg fresh plant samples or 20 mg dried plant samples.

Tissue Lysis: Add 400 µl Buffer A1 and 4 µl RNase A. Incubate in a 65℃ water bath for 10 min.

Removal of impurities such as protein: Add 130 µl Buffer A2 and incubate on ice for 5 min. Centrifuge at 14,000 rpm (18,400 × g) for 5 - 10 min. Collect the supernatant.

Adjustment of the Binding Environment: Add Buffer A3 (1.5 × the volumes of supernatant) to the supernatant and mix thoroughly.

DNA Adsorption: Transfer all the mixture to a FastPure gDNA Columns IV and centrifuge at 12,000 rpm (13,400 × g) for 30 - 60 sec.

Removal of Salt Ions: Add 600 µl Buffer AW, centrifuge at 12,000 rpm (13,400 × g) for 30 sec (twice).

Ethanol Removal: Centrifuge the empty column at 12,000 rpm (13,400 × g) for 2 min.

DNA Elution: Add 50 - 100 µl pre-heated Elution Buffer, incubate at room temperature for 2 min and centrifuge at 12,000 rpm (13,400 × g) for 1 min.

08/Experiment Process

08-1/Sample Processing

Liquid Nitrogen Grinding:

Quickly grind 100 mg of fresh plant samples or 20 mg of dried plant samples into powder in liquid nitrogen. And transfer the powder to a 1.5 ml centrifuge tube.

▲ Do not process more sample than the kit-capacity, as this may result in inadequate sample lysis.The input amount can be increased for samples with high water content, such as strawberries and watermelons.

▲ Store at -85 ~ -65℃ if the ground sample is not used immediately for the next step.

08-2/DNA Extraction

1. Immediately add 400 µl Buffer A1 and 4 µl RNase A to the ground sample powder, vortex and shake.

   ▲ Optional: When the polysaccharide content is particularly high, 2% PVP-40 can be added to Buffer A1. When the polyphenol content is particularly high, 0.2% β-mercaptoethanol can be added to Buffer A1. They can also be added at the same time.

2. Incubate the lysate in the 65℃ water bath for 10 min, inverting the tube 2 - 3 times to facilitate lysis.
3. Add 130 μl Buffer A2 into the above mixture and mix well.Incubate on ice for 5 min, and centrifuge at 14,000 rpm (18,400 × g) for 5 - 10 min. Transfer the supernatant to a new 1.5 ml RNase-free centrifuge tube. Be careful not to absorb interfacial material.
4. Calculate the volume of supernatant. Add Buffer A3 (1.5 × the volumes of supernatant) to the supernatant (check whether absolute ethanol has been added before use) and immediately mix well by pipetting up and down, such as add 750 μl Buffer A3 to 500 μl supernatant.
   ▲ Flocculent precipitation may occur after adding Buffer A3, and the next operation can be carried out after mixing.
5. Transfer the above mixture to a FastPure gDNA Columns IV (already fitted in a Collection Tube). Centrifuge at 12,000 rpm (13,400 × g) for 30 - 60 sec, and discard the filtrate.
   ▲ If the volume of the mixture exceeds 700 μl, centrifuge successive aliquots in the same FastPure gDNA Columns IV. Discard the filtrate after each centrifugation.
6. Add 600 μl Buffer AW (check whether absolute 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 FastPure gDNA Columns IV back into Collection Tube. Centrifuge at 12,000 rpm (13,400 × g) for 2 min to remove the residual Buffer AW from the FastPure gDNA Columns IV.
   ▲ After centrifuging the empty column, air dry the column for 2 - 5 min for the residual ethanol to fully evaporate.
9. Transfer the FastPure gDNA Columns IV into a new 1.5 ml centrifuge tube, and add 50 - 100 μl of Elution Buffer (preheated to 65 ~ 70℃) to the center of the membrane without touching the column. Incubate at room temperature for 3 - 5 min and centrifuge at 12,000 rpm (13,400 × g) for 1 min.
   ▲ The amount of elution volume should be more than 50 μl, and less than 50 μl will lead to a decrease in elution efficiency.
   ▲ Repeat the Elution step with a new Elution Buffer, which may increase the yield but decrease the concentration.
   ▲ For the highest yield, it is recommended to add the first eluent back into theFastPure gDNA Columns IV and repeat the elution step.
10. Discard the FastPure gDNA Columns IV. The extracted DNA can be used directly for downstream experiments or stored at -20℃.

09/FAQ & Troubleshooting

FAQ	Cause	Solution
Clogged FastPure gDNA Columns IV	1. Incomplete grinding of sample	Grind the sample as thoroughly as possible. Mix immediately after adding the Buffer A1.
	2. Lysate too sticky	Reduce the sample input amount.
	3. Small centrifugal speed	Increase centrifugal speed.
No DNA extracted or low DNA yield	1.Too much input and insufficient sample lysis	Decrease input and ensure thorough grinding of the sample.
	2.Improper binding conditions	Accurately estimate the amount of supernatant in 08-2/DNA Extraction/Step 4, adding 1.5 times the volume of Buffer A3 should be accurate.
	3. Residual ethanol on FastPure gDNA Columns IV	Make sure the empty column was centrifuged for 2 min. Please open the lid and air-dry for 2 - 5 min to completely remove the ethanol.
	4. No absolute ethanol is added to Buffer AW	Add the corresponding amount of absolute ethanol.
	5. Incomplete elution	The eluent must be added to the center of the membrane. Increase the elution volume or the number of elution times.
Pigment remains on the DNA solution or column membrane	1.Not enough rinses	Add 500 µl absolute ethanol and rinse again after 08-2/DNA Extraction/Step 7
	2.Too much input	Reduce the sample input amount.
Inhibition of downstream reaction	1. Ethanol contamination	Make sure the empty column was centrifuged for 2 min. Please open the lid and air-dry for 2 - 5 min to completely remove the ethanol.
	2. Exfoliation of silicon matrix membrane components	Centrifuge the eluted gDNA solution again at 12,000 rpm (13,400 × g) for 1 min. Take the supernatant carefully.

Nanjing Vazyme Biotech Co.,Ltd.
Tel: +86 25-83772625
Email: info.biotech@vazyme.com
Web: www.vazyme.com
Loc: Red Maple Hi-tech Industry Park, Nanjing, PRC
Follow Us