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Plant DNA Mini Kit

Product Number: DNK1501

Shipping and Storage

1. Buffer AP1 and AP3/E may experience precipitation and precipitation at low temperatures. They can be re dissolved in a water bath at 65℃ for a few minutes (AP3 can be heated before adding ethanol, not after adding ethanol). After restoring clarity and transparency, they can be cooled to room temperature before use.
2. To avoid volatilization, oxidation, and pH changes caused by prolonged exposure of reagents to the air, each solution should be covered tightly in a timely manner after use.

Components

Component	Storage	DNK1501 50 preps	DNK1502 100 Preps	DNK1503 200 Preps
RNase A(10mg/ml)	-20℃	250μl	500μl	1ml
Buffer AP1	RT	20ml	40ml	80ml
Buffer AP2	RT	7ml	13ml	26ml
Buffer AP3/E	RT	15ml	25ml	50ml
Buffer WB	RT	13ml	25ml	50ml
Buffer EB	RT	15ml	15ml	20ml
Adsorption column AC	RT	50	100	200
Collection tube (2ml)	RT	50	100	200

Description

This reagent kit adopts a DNA adsorption column and a novel unique solution system, suitable for rapid and simple extraction of genomic DNA from plant samples containing phenols, polysaccharides, and enzyme inhibitors. The purification of DNA from one or more 100mg fresh or 20mg dry plant samples can be completed within 30 minutes. The extraction process does not require the extraction of toxic organic compounds such as phenols and chloroform, nor does it require time-consuming isopropanol or ethanol precipitation. It can quickly and efficiently remove impurities such as polysaccharides, phenols, and enzyme inhibitors. The purified DNA can be directly used for PCR, enzyme digestion, and hybridization experiments.

Fresh or dry plant tissues (cells) are ground and then lysed by lysate; Proteins, polysaccharides, and cell debris are precipitated and removed; Then, the genomic DNA is selectively adsorbed onto the silica matrix membrane in a highly dissociated salt state. Through a series of rapid rinsing centrifugation steps, impurities such as polysaccharides, polyphenols, cellular metabolites, proteins, etc. are further removed. Finally, the pure genomic DNA is eluted from the silica matrix membrane using low salt Buffer EB.

Features

1. The silicon matrix membranes inside the centrifugal adsorption column are all made of specially designed adsorption membranes, with minimal differences in adsorption capacity between columns and good repeatability. Overcoming the drawback of unstable membrane quality in domestic reagent kits.
2. No toxic reagents such as phenol are required, and no steps such as ethanol precipitation are required.
3. Fast and simple, the operation of a single sample can generally be completed within 1 hour.
4. Several types of polysaccharides, polyphenols, and multiple column washes ensure high purity, with a typical OD260/OD280 ratio of 1.7-1.9, which can be directly used for PCR, Southern blot, and various enzyme digestion reactions.

Application

Suitable for rapid extraction of plant tissue cells and fungal genomic DNA.

Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a speed of up to 13000 rpm.
2. Preheat the required water bath to 65℃ for later use before starting the experiment.
3. Buffer AP3/E contains irritating compounds, and latex gloves should be worn during operation to avoid contact with skin, eyes, and clothing. If it gets on the skin or eyes, rinse with plenty of water or physiological saline.
4. The amount of DNA extracted from plant tissue materials from different sources may vary, with a typical yield of 3-25μg from 100mg fresh tissue.
5. The Buffer EB does not contain chelating agent EDTA and does not affect downstream enzyme cleavage, ligation, and other reactions. Water can also be used for elution, but it should be ensured that the pH is greater than 7.5, as low pH can affect elution efficiency. Wash DNA with water and store it at -20℃. If DNA needs to be stored for a long time, it can be eluted with TE buffer (10mM Tris HCl, 1mM EDTA, pH 8.0). However, EDTA may affect downstream enzyme digestion reactions and can be diluted appropriately when used.

Protocol (please read the note before the experiment)

Tip:1)Before the first use, please add the specified amount of anhydrous ethanol to Buffer WB, mix well, and mark the added ethanol in the box in a timely manner to avoid multiple additions!

2)Please add the specified amount of anhydrous ethanol to Buffer AP3/E before first use!

1. Take an appropriate amount of plant tissue (100 mg of fresh tissue or 20 mg of dry weight tissue, some more samples can be taken to compensate for the loss of adhesion on the mortar), add liquid nitrogen to the mortar, and grind it thoroughly into fine powder.

   Before grinding, prepare a 1.5ml centrifuge tube and add 400μl Buffer AP1 and 4μl RNase A (10mg/ml) at room temperature for later use.
2. Transfer fine powder (100mg of fresh tissue or 20mg of dry weight tissue) to a previously prepared 1.5ml centrifuge tube (with added 400μl Buffer AP1 and 4μl RNase A (10mg/ml) for vortex oscillation, thoroughly mixing to aid in lysis.
3. 65℃ water bath for 10 minutes, during the water bath process, the centrifuge tube can be reversed 2-3 times to mix the sample.

   Note: This step can also be operated at room temperature, leaving it at room temperature for 10 minutes, but the DNA yield may decrease slightly.
4. Add 130μl Buffer AP2, vortex oscillate and mix for 1 minute, place on ice for 5 minutes, centrifuge at 13000rpm for 5-10 minutes, carefully aspirate the supernatant into a new 1.5ml centrifuge tube, being careful not to aspirate any interfacial substances.

   This step can precipitate and remove various impurities such as proteins, polysaccharides, etc.
5. Calculate the supernatant and add 1.5 times the volume of AP3/E (please check if anhydrous ethanol has been added first!), then shake and mix immediately.

   Adding AP3/E may result in flocculent precipitation, but it does not affect DNA extraction. Please add AP3/E directly to the supernatant and immediately blow or shake to mix well.
6. Add the mixture obtained from the previous step (including possible precipitation) to an adsorption column AC, centrifuge at 13000 rpm for 30-60 seconds (with the adsorption column placed in the collection tube), and discard the waste liquid in the collection tube (centrifuge with 650μl first, discard the waste liquid, then add the remaining solution and centrifuge again).
7. Add 600μl Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 13000rpm for 30 seconds, and discard the waste liquid.
8. Repeat step 7.

   Note:If the adsorption column membrane still shows a lot of green pigment, this step of rinsing can be added. Add 500μl of anhydrous ethanol to the adsorption column AC, centrifuge at 13000rpm for 30 seconds, and discard the waste liquid.
9. Put the adsorption column AC back into the empty collection tube, centrifuge at 13000rpm for 2 minutes, and try to remove Buffer WB as much as possible to avoid residual ethanol in Buffer WB inhibiting downstream reactions.

10. Take out the adsorption column AC and place it in a clean centrifuge tube. Add 50μl-100μl Buffer EB to the middle of the adsorption membrane and let it stand at room temperature for 3-5 minutes. Centrifuge at 13000rpm for 1 minute. Add the obtained solution back into the adsorption column, let it stand at room temperature for 2 minutes, and centrifuge at 13000 rpm for 1 minute.

    The larger the elution volume, the higher the elution efficiency. If a high yield is expected and required, the elution volume can be increased. If a high DNA concentration is required, the elution volume can be appropriately reduced, but the minimum volume should not be less than 50μl. If the volume is too small, the DNA elution efficiency can be reduced and DNA yield can be reduced.

11. DNA can be stored at 2-8℃, and if it needs to be stored for a long time, it can be placed at -20℃.