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Plant Max Genomic DNA Kit

Product Number: DNK3801

Shipping and Storage

1. When Buffer AP1 and AP3/E are at low temperatures, precipitation and precipitation may occur. They can be dissolved again in a water bath at 65°C for a few minutes (Buffer AP3/E can be heated before adding ethanol, but 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	DNK3801 10 Preps
RNase A(10mg/ml)	-20°C	500u1
Buffer AP1	RT	50ml
Buffer AP2	RT	20ml
Buffer AP3/E	RT	40ml
Buffer WB	RT	25ml×2
Buffer EB	RT	20ml
Adsorption column AC	RT	10
Collection tube (50ml)	RT	10

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 fresh or 200mg dry plant samples can be completed in about 1 hour. 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 alcohol inhibitors. The purified DNA can be directly used for PCR, alcohol cleavage, and hybridization experiments.

Fresh or dry plant tissues (cells) are ground and then lysed in the lysate, and 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, pure genomic DNA is eluted from the silica matrix membrane in a low salt elution buffer.

Features

1. The silicon matrix membrane inside the centrifugal adsorption column is entirely made of imported 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 alcohol cleavage reactions.

Application

Suitable for rapid extraction of genomic DNA from plant tissues, cells, and fungi

Note

1. Preheat the required water bath to 65°C for later use before starting the experiment.
2. 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
3. The amount of DNA extracted from plant tissue materials from different sources may vary, and the typical yield of fresh tissue can reach 30-260ug.
4. Buffer EB does not contain chelating agent EDTA and does not affect downstream reactions such as alcohol cleavage and connection. 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. DNA washed with water should be stored at -20°C. If DNA needs to be stored for a long time, it can be eluted with TE buffer (10mM Tris HCl, lmM EDTA, pH 8.0), but EDTA may affect downstream fermentation reactions and can be diluted appropriately when used.

Protocol(Please read the precautions before the experiment)

Tips: 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!
3) Preheat Buffer AP1 in a 65°C water bath.

1. Take an appropriate amount of plant tissue (with a maximum processing capacity not exceeding 1g of fresh tissue or 200mg of dry weight tissue) and add liquid nitrogen to a mortar to grind thoroughly into fine powder.
2. Transfer fine powder to a 15ml centrifuge tube, do not thaw, add 5ml Buffer AP1 (preheated at 65°C) and 40ul RNase A (10mg/ml), vortex oscillate, mix well to aid in cracking.
   If tissue lysis is difficult, a 10 second gentle kneading step can be added as needed to aid in lysis. In most cases, centrifugation is not necessary to remove partially lysed tissue, as there is a subsequent step of centrifugation removal.
3. **Optional steps:** When the polysaccharide content is particularly high, 2% PVP40,000 can be added to Buffer AP1; When the polyphenol content is particularly high, 0.2% beta mercaptoethanol can be added to Buffer AP1. Both can also be added simultaneously.
4. 65°C water bath for 10-15 minutes, invert the centrifuge tube 2-3 times during the water bath process, and mix the sample.
5. Add 1.8ml Buffer AP2, mix well, let it stand on ice for 10 minutes, centrifuge at 9000×g room temperature for 10 minutes, carefully aspirate the supernatant into a new 50ml centrifuge tube, being careful not to aspirate any interface substances.
   If there is no high-speed centrifuge, it is also possible to centrifuge 4000-5000×g and extend the time appropriately.
6. Calculate the amount of supernatant, add 1.5 times the volume of Buffer AP3/E (please check if anhydrous ethanol has been added first!), and immediately vortex and shake to mix well.
   Adding Buffer AP3/E may result in precipitates, but it does not affect DNA extraction. Please add Buffer AP3/E directly to the supernatant and immediately vortex oscillate and mix well.
7. Add the mixture obtained from the previous step (including possible precipitates) to an adsorption column AC, (place the adsorption column into a collection tube) centrifuge 3000-5000×g for 5 minutes, and discard the waste liquid in the collection tube.
8. Add 10m1 Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge 3000-5000×g for 4 minutes, and discard the waste liquid.
9. Add 10m1 Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge 3000-5000×g for 3 minutes, and discard the waste liquid.
10. Put the adsorption column AC back into the empty collection tube, centrifuge at the highest speed (preferably greater than 9000×g, if the centrifuge speed is low, the centrifugation time needs to be extended accordingly) for 10 minutes to dry the residual ethanol in the membrane matrix. Use the nozzle to remove any residual ethanol between the inner ring pressure ring and the column wall, and air dry at room temperature or in an oven for a few minutes.
11. Take out the adsorption column AC and place it in a clean centrifuge tube. Add 1-2ml of Buffer EB in the middle of the

adsorption area (Buffer EB can be preheated in a 65-70°C water bath in advance), and let it stand at room temperature for 5 minutes. Centrifuge 3.000-5.000×g for 3 minutes to obtain DNA. In addition, the obtained solution can be re added to the adsorption column, left at room temperature for 2 minutes, and then eluted again to increase concentration and yield.

The larger the elution volume, the higher the elution efficiency. If a higher yield is expected and required, the elution volume can be increased. If a higher DNA concentration is required, the elution volume can be appropriately reduced, but the minimum volume should not be less than 500ul. If the volume is too small, the elution efficiency of DNA can be reduced to reduce DNA production

12. DNA can be stored at 2-8°C, and if stored for a long time, it can be stored at -20°C.