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CTAB Max Plant DNA Kit

Product Number: DNK4401

Shipping and Storage

1. Buffer PL or Buffer IR may precipitate and precipitate at low temperatures. It can be dissolved again by taking a water bath at 55℃ for a few minutes, restoring clarity and transparency, and then cooled to room temperature before use. The concentration of Guanidine Hydrochloride in Buffer PQ is high, and there may be some precipitation after adding ethanol, which does not affect its use. Take the supernatant directly and use it.
2. Avoid volatilization, oxidation, and pH changes caused by prolonged exposure of reagents to the air. Each solution should be covered tightly after use.

Components

Component	Storage	DNK4401 10 Preps
Buffer PL	RT	100 ml
Buffer PQ	RT	30 ml×2
Buffer IR	RT	100 ml
Buffer WB	RT	25 ml×2
Buffer EB	RT	15 ml×2
Adsorption column AC	RT	10
Collection tube (50ml)	RT	10

Description

The improved classic CTAB plant DNA extraction solution (with the addition of various polysaccharide and polyphenol removal components tailored to plant characteristics) rapidly lyses cells and inactivates intracellular nucleases. After chloroform extraction, polysaccharides, polyphenols, and proteins are removed by centrifugation (as needed, isopropanol is also added to the supernatant to precipitate genomic DNA and further remove other impurities), 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 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. The typical OD260/OD280 ratio ranges from 1.7 to 1.9, with a length of up to 30kb-50kb. It can be directly used for PCR, Southern blot, and various enzyme digestion reactions.

Application

Suitable for rapid extraction of plant genomic DNA

Note

For Research Use Only

1. All centrifugation steps are completed at room temperature, using a desktop centrifuge with a rotational speed of 9000×g, which can accommodate 50ml centrifuge tubes.
2. Preheat the required water bath to 65℃ for later use before starting the experiment.
3. We need to provide chloroform/isoamyl alcohol (mixed in a volume ratio of 24:1), anhydrous ethanol, and β- Mercaptoethanol.
4. Buffer PQ and Buffer IR contain 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.
5. 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.
6. Buffer EB does not contain chelating agent EDTA and does not affect downstream enzyme cleavage, linking, 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 precautions before the experiment)

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

2)Take the appropriate amount of Buffer PL and preheat it at 65℃. Before use, add β-mercaptoethanol to the final concentration of 2%.

1. Take about 1-2g of fresh plant tissue or about 0.3-0.4g of dry weight tissue, add liquid nitrogen and thoroughly grind into fine powder.
2. Transfer the fine powder to a 50ml centrifuge tube, do not thaw, add 10ml of 65℃ preheated Buffer PL (confirm that B-mercaptoethanol has been added to 2%), vigorously vortex and shake well, and gently blow with a large caliber gun to aid in cracking.

   If tissue lysis is difficult, a gentle 10 second step of homogenization can be added as needed to assist in lysis.
3. 65℃ water bath for 30-60 minutes, invert the centrifuge tube to mix the sample several times during the water bath process.

   Optional: If the organization is dry or the yield is low, the water bath time can be appropriately extended. If there is a lot of RNA residue, 100μl RNA enzyme (20mg/ml) can be added before the water bath.

   Note: If the extracted DNA contains a large amount of residual RNA, resulting in trailing bands, twisted bands, and high background during electrophoresis, which is abnormal for electrophoresis, the RNA can be digested by adding 1% RNA enzyme (10mg/ml) at 37℃ or room temperature for half an hour. After digestion, it can be used for PCR or enzyme digestion without special treatment.

   Add 10ml of chloroform or chloroform/isoamyl alcohol (mixed in a volume ratio of 24:1), invert and mix thoroughly for a few minutes (or vortex mix well), centrifuge for 10 minutes above 9000×g.

   If the extracted plant tissue is rich in polysaccharides and polyphenols, it can be extracted once with an equal volume of phenol/chloroform (1:1) before step 4.
4. Carefully aspirate the supernatant into a new 50ml centrifuge tube, being careful not to aspirate any interfacial substances.

   If the supernatant is cloudy, repeat step 4 until a clear supernatant is obtained.
5. Accurately estimate the amount of supernatant, add 1.5 times the volume of Buffer PQ (please check if anhydrous ethanol has been added first!), then immediately vortex and mix thoroughly. At this point, precipitation may occur, but it does not affect the experimental results.
6. Add the mixture obtained from the previous step (including possible precipitation) to an adsorption column AC, let it stand for 2 minutes (place the adsorption column in the collection tube), centrifuge 9000×g for 2 minutes, and discard the waste liquid in the collection tube (centrifuge up to 20ml of the mixture each time, discard the waste liquid, add the remaining solution, and centrifuge again).
7. Add 10ml of Buffer IR, centrifuge 9000×g for 2 minutes, and discard the waste liquid.

8. Add 10ml of Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 9000 × g for 2 minutes, and discard the waste liquid
9. Repeat step 8 once.
10. Place the adsorption column AC back into the empty collection tube and centrifuge at the highest speed (preferably greater than 9000×g, if the centrifuge speed is low, the centrifuge time needs to be extended accordingly) for 10-15 minutes to dry the residual ethanol in the membrane matrix. Use the nozzle to remove any residual ethanol between the inner ring and the column wall, and air dry at room temperature or in an oven for a few minutes. The purpose of this step is to completely remove residual ethanol from the adsorption column, which inhibits downstream reactions and severely reduces the elution efficiency, Reduce DNA production. If the elution yield is low, step 12 must be added.
11. Optional steps: Choose one of the following two methods to dry the column:
    11.1. Remove the column and place it in a vacuum container, seal the vacuum container, and provide vacuum for 15 minutes;
    11.2. Place the column in a vacuum drying oven or oven at 60-65℃ for 10-15 minutes.
12. Take out the adsorption column AC and place it in a clean centrifuge tube. Add 1.5-2ml of Buffer EB to the middle of the adsorption membrane (Buffer EB can be preheated in a 65-70℃ water bath for better results). Leave it at room temperature for 3-5 minutes and centrifuge 9000×g for 4-5 minutes. If a large amount of DNA is required, the obtained solution can be re added to the centrifuge adsorption column and centrifuged for 2 minutes.

    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 1ml. If the volume is too small, the DNA elution efficiency can be reduced and DNA production can be reduced.
13. DNA can be stored at 2-8℃, and if it needs to be stored for a long time, it can be stored at -20℃