MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

CTAB Plant Genomic DNA Kit

Product Number: DNK1401

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	DNK1401 50 Preps	DNK1402 100 Preps	DNK1403 200 Preps
Buffer PL	RT	30 ml	60 ml	120 ml
Buffer PQ	RT	18 ml	35 ml	70 ml
Buffer IR	RT	25 ml	50 ml	100 ml
Buffer WB	RT	13 ml	25 ml	50 ml
Buffer EB	RT	15 ml	15 ml	20 ml
Adsorption column AC	RT	50	100	200
Collection tube (2ml)	RT	50	100	200

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 and polyphenols are removed, and multiple column washes are used to ensure high purity. The typical OD260/OD280 ratio is 1.7-1.9, with a length of up to 20kb-50kb. It can be directly used for PCR, Southern blot, and various enzyme digestion reactions.

Application

Suitable for rapid extraction of plant tissues (including complex polysaccharide and phenolic plants) and fungal genomic DNA.

Note

For Research Use Only

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a speed of up to 13000rpm.
2. Preheat the required water bath to 65℃ for later use before starting the experiment.
3. We need to provide chloroform, anhydrous ethanol, and β-mercaptoethanol ourselves.
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 Buffer TE (10mM Tris HCl, 1mM EDTA, pH 8.0), but EDTA may affect downstream enzyme digestion reactions and can be diluted appropriately when used.
7. This reagent kit is prepared according to the standard extraction process for each solution volume. If the sample DNA content is low or the yield is low, the extraction amount needs to be increased, and an additional solution needs to be purchased.

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 100mg of fresh plant tissue or about 30mg of dry weight tissue, add liquid nitrogen and thoroughly grind into fine powder.
2. Quickly transfer the ground powder to a centrifuge tube pre filled with 600μl of 65℃ preheated Buffer PL (before the experiment, add mercaptoethanol to the preheated PL to achieve a final concentration of 2%), quickly invert and mix, then place the centrifuge tube in a 65℃ water bath for 20-30 minutes. During the water bath process, invert the centrifuge tube several times to mix the sample.

Optional steps: If it is expected that the sample RNA is abundant and prone to residue, 5-6μl of RNA enzyme (20mg/ml) can be added before the water bath. If the organization is dry or the yield is low, the water bath time can be appropriately extended.

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, 1% RNA enzyme (10mg/ml) can be added to 37℃ or left at room temperature for half an hour to digest the RNA. After digestion, it can be used for PCR or enzyme digestion without special treatment.

3. Add 700μl of chloroform, mix well, and centrifuge at 13000 rpm for 5 minutes.

Optional steps (generally not required): If extracting plants rich in polysaccharides, polyphenols, or starch, Tris saturated phenol (pH 8.0)/chloroform (1:1) can be used to extract once before step 3.

4. Carefully aspirate the supernatant (about 600μl) into a new 1.5ml centrifuge tube (be careful not to aspirate any interfacial substances). Add 1.5 times the volume of Buffer PQ (please check if anhydrous ethanol has been added first!), then immediately vortex and mix thoroughly.
5. Transfer the mixed liquid into the adsorption column AC, centrifuge at 13000 rpm for 30 seconds, and discard the waste liquid. (The adsorption column has a volume of about 700μl and can be added to centrifugation in stages.)
6. Add 500μl of Buffer IR, centrifuge at 13000 rpm for 30 seconds, and discard the waste liquid.
7. Add 600μl of Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 13000 rpm for 30 seconds, and discard the waste liquid.
8. Repeat step 7.

9. Return the adsorption column AC to the empty collection tube and centrifuge at 13000rpm for 2 minutes. Try to remove Buffer WB as much as possible to prevent residual ethanol from inhibiting downstream reactions.
10. Take out the adsorption column AC and place it in a clean centrifuge tube. Add 50-100μl of Buffer EB to the middle of the adsorption membrane and let it sit at room temperature for 3-5 minutes. Centrifuge at 13000rpm for 1 minute. Add the obtained solution back into the centrifugal adsorption column, let it stand at room temperature for 2 minutes, and centrifuge at 13000 rpm for 1 minute.

Note:1)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 production can be reduced.

2)If pursuing maximum production, preheating the elution buffer in a water bath at 80-100℃ before adding can increase production.

Appendix (protocol for operating samples with low DNA content or low yield):

1. Take an appropriate amount of plant tissue (fresh tissue 400mg or dry weight tissue 200mg) and add liquid nitrogen to a mortar to grind it into fine powder.
2. Transfer the fine powder to a 15ml centrifuge tube, do not thaw, add 9ml of 65℃ preheated Buffer PL (confirm that β-mercaptoethanol has been added to 2%), vigorously vortex and shake well, and use a large caliber gun to blow to aid in cracking.

Note:If tissue lysis is difficult, a gentle 10 second step of homogenization can be added as needed to assist in lysis.

3. Leave at room temperature for 60 minutes, occasionally invert the centrifuge tube to mix the sample several times.

Note:If the organization is dry or the yield is low, it can be placed in a 65℃ water bath.

4. Add 4.5ml of chloroform, vortex thoroughly and mix well. Centrifuge 3000g for 10 minutes.
5. Carefully aspirate the supernatant into a new 15ml centrifuge tube, being careful not to aspirate any interfacial substances. Repeat step 4.
6. Carefully aspirate the supernatant into a new 15ml centrifuge tube, estimate the amount of supernatant, add 0.7 times the volume of isopropanol, vortex and mix well to precipitate DNA.
7. Immediately centrifuge 3000g for 20 minutes to precipitate DNA, discard the supernatant, invert the centrifuge tube and place it on a tissue to control the residual liquid. Carefully use a pipette to absorb the residual liquid around the precipitate (do not dry the DNA precipitate too dry).
8. Add 300μl-400μl of sterilized water preheated to 65℃ to dissolve DNA again. It may be necessary to briefly incubate at 65℃ to aid in dissolution, and continuously gently tap the bottom of the tube to aid in dissolution.
9. Add 1.5 times the volume of Buffer PQ (450μl-600μl, please check if anhydrous ethanol has been added!), then immediately vortex and mix thoroughly.
10. The subsequent steps are exactly the same as those in step 5 above.