Plant DNA Extraction Kit

(Silicone Membrane Centrifugal Column Method)

Cat. No./Spec.: N1221/24 preps; N1222/100 preps

Kit Contents

Component	N1221	N1222
Buffer PAL	20 ml	80 ml
Buffer BDP	20 ml	80 ml
Buffer GWP	20 ml	110 ml
Buffer SW2	30 ml	110 ml
Elution Buffer	10 ml	20 ml
DNA Spin Column	24 pcs	100 pcs
Collection Tube	24 pcs	100 pcs

Description

This product combines the CTAB extraction method with column purification technology, suitable for extracting high-purity DNA from various plants and fungal samples. The obtained DNA can be directly used for PCR, SSR, AFLP, RAPD, and Southern blot experiments. The kit provides a less toxic substitute for chloroform (Buffer BDP) and uses an alcohol-free, high-concentration guanidine salt-mediated filtration purification to efficiently remove polysaccharides, polyphenols, and other metabolites and RNA from the samples.

Storage

This product can be stored at room temperature (15~25°C) for 18 months. Buffer PAL may precipitate at low temperatures, which can be dissolved by incubating at 55°C and mixing thoroughly. The product uses PET reagent bottles, which should not be subjected to high-temperature, high-pressure sterilization or direct incubation above 55°C.

Protocol

A. Routine Samples

1. Sample grinding: Grind plant/fungal samples into powder with liquid nitrogen, transfer 50~150mg fresh/frozen samples or 15~40mg dried samples to a 2ml centrifuge tube.

• Correct sample amount is essential for ideal results. Excessive samples can cause column blockage, leading to reduced yield and purity. Due to the large variation in DNA and metabolite content in plant samples, it is recommended to use 50mg fresh or 15mg dried samples for the first experiment and adjust the sample amount based on the results. For samples rich in mucilage, it is recommended not to exceed 30~50mg (fresh) sample amount.

• Increase sample amount: The maximum binding capacity of the purification column is 20μg. If the DNA content in the sample is low, the sample amount can be increased by 2~3 times, and Buffer PAL and Buffer BDP can be added proportionally, then purified by multiple column passes.

2. Sample lysis: Immediately add 700μl preheated to 65°C Buffer PAL to the sample, vortex vigorously to fully disperse the sample, and incubate in a 65°C water bath for 15~30 minutes, mixing 2~3 times during the process.

• Add β-mercaptoethanol to Buffer PAL to a final concentration of 2% before the experiment to improve the antioxidant capacity of the lysis solution. Since β-mercaptoethanol has a strong odor, most samples do not require addition. Buffer PAL is a CTAB lysis solution, and more reagents can be self-prepared or ordered. PVP-40 must not be added to Buffer PAL or self-prepared CTAB lysis solution in this step.

3. Organic extraction: Add 700μl Buffer BDP (or chloroform), vortex mix for 15 seconds. Centrifuge at 12,000 x g at room temperature for 5 minutes.

• For tissue samples rich in polyphenols or starch, an additional step of phenol-chloroform equal volume extraction can be added before step 3 to remove polysaccharides and other impurities. Buffer BDP is a substitute for chloroform, with the main component being less toxic bromochloropropane. In case of skin contact, immediately remove contaminated clothing, rinse with plenty of running water, and seek medical attention.

4. Adjust binding conditions (high salt-mediated): Transfer 600μl supernatant to a new centrifuge tube. Add 600μl Buffer GWP, invert and mix 6-8 times.

• High salt mediation: Purification column B10 only adsorbs DNA, not RNA, under guanidine (GWP) conditions, eliminating the need for RNase treatment. However, the maximum adsorption capacity of the column is only 10μg, and excess DNA will flow out with the filtrate. High salt mediation effectively removes pigments, polysaccharides, and proteins, and other metabolic products.

• Alcohol-mediated: If the DNA content in the sample exceeds 10μg, ethanol mediation can be used to increase yield. Transfer 600μl supernatant to a new centrifuge tube, add 10μl RNase A (self-prepared), invert and mix, and let it stand at room temperature for 10 minutes. Add 300μl Buffer GWP and 600μl anhydrous ethanol, invert and mix 15-30 times, and proceed to step 5.

5. Column purification: Place the purification column in a collection tube, transfer half the volume of the mixture to the column. Centrifuge at 10,000 x g for 1 minute. Discard the filtrate in the collection tube and replace the column, transfer the remaining mixture to the column, and centrifuge again.

6. Removal of proteins and RNA: Discard the filtrate, replace the column in the collection tube, add 400μl Buffer GWP, and centrifuge at 10,000 × g for 1 minute.

7. Desalination: Discard the filtrate, replace the column in the collection tube, add 750μl Buffer SW2, and centrifuge at 10,000 × g for 1 minute.

• If the DNA concentration is below 50ng/μl, it is recommended to divide Buffer SW2 into two portions, each 500μl, to stabilize the A260/230 ratio.

8. Discard the filtrate, replace the column in the collection tube. Centrifuge at 10,000 x g for 1 minute to remove residual ethanol from the column.

9. Elution: Transfer the column to a new 1.5ml centrifuge tube, add 50μl preheated to 65°C Elution Buffer to the center of the column membrane. Let it stand at room temperature for 3 minutes, and centrifuge at 10,000 x g for 1 minute.

10. Re-elution: Add another 50μl of preheated to 65°C Elution Buffer to the center of the column membrane. Let it stand for 3 minutes. Centrifuge at 10,000× g for 1 minute.

11. Discard the DNA binding column, and store the DNA at -20°C.

B. Difficult-to-extract Plant Samples

For samples with low nucleic acid content or difficult extraction, add PVP-40 and β-mercaptoethanol to Buffer PAL to improve lysis and antioxidant capacity. Add 50μl of 40% PVP-40 (can also be replaced with PVP-40 powder) and 20μl of β-mercaptoethanol per 1ml Buffer PAL. This mixture can be stored at room temperature for one month.

1. Sample grinding and lysis: Grind plant/fungal samples into powder with liquid nitrogen, transfer an appropriate amount of fresh/frozen or dried samples to the corresponding centrifuge tubes, immediately add the preheated to 65°C Buffer PAL/PVP-40 mixture, vortex to fully disperse the sample, and incubate in a 65°C water bath for 20 minutes, mixing 2~3 times during the process.

• Buffer PAL usage: Add 0.7ml Buffer PAL per 100mg fresh or 20mg dried sample. If the nucleic acid content in the sample is low or more DNA is required, the sample amount can be increased to 200-500mg, and Buffer PAL can be added proportionally. More Buffer PAL can be self-prepared or ordered.

2. Organic extraction: Add an equal volume of Buffer BDP or chloroform, vortex mix for 15 seconds. Centrifuge at 10,000 x g at room temperature for 5 minutes.

• For samples rich in polyphenols or starch, an additional step of phenol-chloroform equal volume extraction can be added before step 2 to remove impurities. Buffer BDP is a substitute for chloroform, with the main component being less toxic bromochloropropane. In case of skin contact, immediately remove contaminated clothing, rinse with plenty of running water, and seek medical attention. More Buffer BDP can be ordered separately. If the total volume exceeds 2ml, perform the operation in a 5-15ml centrifuge tube, and change the centrifugation conditions to 4,000~5,000 x g for 15 minutes.

3. Precipitation enrichment: Transfer the supernatant to a new centrifuge tube, add 0.7 times the volume of isopropanol, gently invert and mix 15~20 times. Centrifuge at 10,000 x g at room temperature for 5 minutes, carefully discard all supernatant, and retain the precipitate.

• If the total volume exceeds 2ml, perform the operation in a 5-15ml centrifuge tube, and change the centrifugation conditions to 4,000~5,000 x g for 10 minutes. When DNA is abundant, DNA forms silky or fibrous precipitates when mixed with isopropanol. Since polysaccharides also form flocculated precipitates, if a lot of precipitate is produced at this step, it indicates that the sample is rich in polysaccharides, and phenol-chloroform equal volume extraction should be performed in the second operation at step 2. If no obvious precipitate is observed when mixing with isopropanol, the sample can be placed at -20°C overnight before centrifugation to collect DNA and improve DNA yield.

4. Dissolution: Briefly centrifuge, and remove all residual liquid. Add 200μl Elution Buffer or sterile water, gently agitate and incubate at 65°C for 10-15 minutes to dissolve DNA.

• Crude genomic DNA obtained by other methods can also be purified with this method. Supplement DNA with water to 250μl, and proceed to step 6.

5. Further purification by column: Add 400μl Buffer GWP to the sample, invert and mix several times. Follow steps 5-11 of procedure A.

• For plant samples rich in polysaccharides, the DNA sample may have obvious clumps. Incubate at 50°C for an additional 10 minutes, and use a pipette to repeatedly aspirate and disperse the gel to release DNA from the gel as quickly as possible. If the total amount of DNA exceeds 10μg, add 0.2ml isopropanol, invert and mix, and then follow step 5 of procedure A for column purification.

OD Value Measurement and Yield

λ A260/280 ratio: The normal value for this product is 1.70~1.90.

λ A260/230 ratio: The normal value for this product is 1.1~2.4. If the nucleic acid concentration is below 50ng/μl, values below this range are also acceptable.

This product is for scientific research use only.