

Complex Polysaccharides-Polyphenols/Fungi Plant DNA Kit

Product Number: DNK55

Shipping and Storage

1. Cracking solution PF may precipitate and precipitate at low temperatures in winter. It can be dissolved again in a 55°C water bath for a few minutes to restore clarity and transparency. After that, it can be gently and thoroughly mixed and cooled to room temperature before use.
2. To avoid the volatilization, oxidation, and pH changes of reagents exposed to air for a long time, the lid of each solution should be promptly closed after use.

Components

Component	Storage	DNK5501 50 Preps	DNK5502 100 Preps	DNK5503 200 Preps
RNase A	4°C	250μL	500μL	1mL
Buffer PF	RT	30mL	60mL	120mL
Buffer PQ	RT	18mL	35mL	70mL
Add the specified amount of ethanol according to the bottle label instructions before the first use				
Buffer PE	RT	16mL	32mL	64mL
Add the specified amount of ethanol according to the bottle label instructions before the first use				
Buffer WB	RT	13mL	25mL	50mL
Add the specified amount of ethanol according to the bottle label instructions before the first use				
Buffer EB	RT	10mL	10mL	20mL
Adsorption column AC	RT	50	100	200
Collection tube	RT	50	100	200

Note: This reagent kit can be stored at room temperature for 12 months without affecting its effectiveness.

Description

This product uses our company's unique complex plant/fungal sample DNA extraction solution (with the addition of various polysaccharides, polyphenols, and starch removal components specific to plant/fungal characteristics) to rapidly lyse cells and inactivate intracellular nucleases. After chloroform extraction, polysaccharides, polyphenols, and protein impurities are removed by centrifugation. Then, DNA is selectively adsorbed onto the silica matrix membrane in a highly ionized salt state. After multiple washes, polysaccharides, polyphenols, cellular metabolites, proteins, and other impurities are further removed. Finally, a low salt elution buffer is used to elute pure DNA from the silica matrix membrane.

Features

1. All the silicon matrix membranes in the centrifugal adsorption column are made of specially designed adsorption membranes, with minimal differences in adsorption capacity between columns and good repeatability. Overcoming the drawbacks of unstable membrane quality in domestic reagent kits.
2. Widely applicable, it can extract the vast majority of samples with high quality, including the most complex polysaccharide polyphenols and starch seed samples.
3. Easy to operate, a single sample operation can generally be completed in 30 minutes.
4. Several methods of removing polysaccharides and polyphenols, as well as multiple column rinses, ensure high purity. The

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typical ratio of OD260/OD280 is 1.7-1.9, and the length can reach 20-50kb. It can be directly used for PCR, Southern blot, and various enzyme digestion reactions.

Note

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°C before starting the experiment.
3. Need to prepare chloroform (or use chloroform substitute), anhydrous ethanol, and β - mercaptoethanol.
4. The amount of DNA extracted from plant tissue materials from different sources may vary, with a typical yield of 3-25 μ g for 100mg fresh tissue.
5. The Buffer EB does not contain chelating agent EDTA, which does not affect downstream enzyme digestion, ligation and other reactions. Water can also be used for elution, but it should be ensured that the pH is greater than 7.5. If the pH is too low, it will affect the 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 washed with TE buffer (10mM Tris HCl, 1mM EDTA, pH 8.0), but EDTA may affect downstream enzyme digestion reactions and can be diluted appropriately when used.
6. This reagent kit is configured 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 additional solutions need to be purchased.

Protocol(Please read the precautions before the experiment)

Note:1)Before use, please add anhydrous ethanol to the buffer PE, buffer PQ, and buffer WB bottles. Please refer to the labels on the bottles for the added volume!

2)Take 600 μ L of buffer PF into a centrifuge tube (if there is precipitation or precipitation at low temperature, it needs to be dissolved again in a 65°C water bath), and add 2% β - mercaptoethanol (600 μ L PF plus 12 μ L β - mercaptoethanol) to buffer PF. Preheat in a 65°C water bath after reversing and mixing. Prepare multiple samples by enlarging them in proportion.

1. Take an appropriate amount of plant tissue (100 mg fresh tissue or 30 mg dry weight tissue, and take some more samples to compensate for the loss of sticking to the mortar) and add liquid nitrogen to the mortar to grind it into a fine powder.
2. Quickly transfer the ground powder to a centrifuge tube pre filled with 600 μ L of 65°C preheated cracking solution PF (add β - mercaptoethanol to the preheated PF before the experiment to make the final concentration 2%), quickly invert and mix, add 5 μ L RNase A (10 mg/mL), and place the centrifuge tube in a 65°C water bath for 20-30 minutes. During the water bath process, invert the centrifuge tube several times to mix the sample.
 - 2.1. If the organization is dry or the yield is low, the water bath time can be appropriately extended to 1 hour.
 - 2.2. If the extracted DNA contains a large amount of residual RNA, resulting in abnormal electrophoresis conditions such as band tailing, band distortion, and high background, 1% RNase A (10 mg/ml) can be added and the RNA can be digested at 37°C or room temperature for 20 minutes. After digestion, it can be directly used for PCR or enzyme digestion without special treatment.
3. Add 700 μ L chloroform, mix well, centrifuge at 13000 rpm for 5 minutes.
 - 3.1. Optional steps (generally not required): If extracting plants rich in polysaccharides, polyphenols, or starch, a volume of Tris saturated phenol (pH 8.0)/chloroform (1:1) can be used for extraction before step 3.
4. Carefully aspirate the supernatant (approximately 600 μ L) into a new centrifuge tube. Add 1.5 times buffer PQ (please check if anhydrous ethanol has been added first!)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 700 μ L and can be added in stages for centrifugation.)
6. Add 500 μ L to buffer PE (please check if anhydrous ethanol has been added first!)Centrifuge at 13000 rpm for 30 seconds and discard the waste liquid.
7. Add 600 μ L 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 13000 rpm for 2 minutes. Try to remove the rinse solution as much as possible to prevent residual ethanol in the rinse solution from inhibiting downstream reactions.
10. Remove the adsorption column AC and place it in a clean centrifuge tube. Add 50-100μL of elution buffer EB to the middle of the adsorption membrane, let it stand at room temperature for 3 minutes, and centrifuge at 13000 rpm 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.
- 10.1. The larger the elution volume, the higher the elution efficiency. If a higher DNA concentration is required, the elution volume can be appropriately reduced. However, it is important to note that a smaller volume can reduce elution efficiency and decrease DNA production (the minimum should not be less than 30μL).
- 10.2. To achieve the highest yield, preheating the elution buffer in an 80-100°C water bath before adding can increase the yield.

Appendix (Operating Procedures for Low DNA Content or Low Yield Samples)

1. Take an appropriate amount of plant tissue (500 mg fresh tissue or 200 mg dry weight tissue) and add liquid nitrogen to a mortar to grind it thoroughly into a fine powder.
 - 2.1. Transfer the fine powder to a 15mL centrifuge tube, do not thaw, add 9mL of buffer PF preheated at 65°C (confirm that β-mercaptoethanol has been added to the final concentration of 2%), vigorously vortex and mix, and use a large caliber gun tip to blow and aid in cracking.
2. If tissue lysis is difficult, a gentle 10 second homogenization step can be added as needed to assist in lysis.
 - 3.1. Leave at room temperature for 1 hour, occasionally invert the centrifuge tube to mix the samples several times.
3. If the organization is dry or the yield is low, it can be placed in a 65°C water bath.
4. Add 4.5mL of chloroform, vortex thoroughly, centrifuge at 3000 × g for 10 minutes.
5. Be careful to transfer the supernatant into a new 15ml centrifuge tube, and be careful not to suck any interfacial substances. Repeat step 4.
6. Carefully transfer the supernatant into a new 15mL centrifuge tube, estimate the amount of supernatant, add 0.7 times the volume of isopropanol, vortex and mix to precipitate DNA.
7. Immediately centrifuge at 3000 × g for 20 minutes to precipitate DNA, discard the supernatant, invert the centrifuge tube and place it on a tissue to dry the residual liquid, and 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°C and 3μL of RNase to dissolve the DNA again. It may be necessary to briefly incubate at 65°C to assist with dissolution, while continuously tapping 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 first!) Immediately vortex and mix thoroughly.
10. The subsequent steps are exactly the same as the standard operating step 5 above.