N96 DNAsecure Plant Kit

Fast and high throughput purification of high quality DNA from various fresh plant tissues

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N96 DNAsecure Plant Kit

(N96 Plate)

Cat. no. 4992718

Kit Contents

Contents	4992718 (2 plates)
Buffer LP1	100 ml
Buffer LP2	40 ml
Buffer LP3	84 ml
Buffer PW	50 ml
Buffer TE	60 ml
RNase A (10 mg/ml)	1.25 ml
N96 Filtration Plate (H)	2
N96 Plate CB3 (H)	2
N96 Well Plate	6
Plate Cover	12
Handbook	1

Storage

This kit can be stored for 12 months in a dry environment under room temperature (15-25°C). For longer storage, please store at 2-8°C. If a precipitate has formed in buffer under 2-8°C, please place the buffer at room temperature or warm at 37°C for 10 min to dissolve the precipitate.

Introduction

This kit adopts a specific DNA binding spin column and unique buffer system, which enables the extraction of genomic DNA from multiple plant tissues. The silicon substrate material adopted in the N96 Plate CB3 (H) is a new material of our company, which allows the column to absorb DNA efficiently and specifically, and to remove protein as much as possible. The genomic DNA extracted by this kit is integrate, pure and stable.

The extracted gDNA can be used for various downstream experiments including enzyme digestion, PCR, library construction and Southern blot,etc.

Product Features

Simple and fast: Ultra-pure gDNA can be obtained within 1 h.

Wide sample types: It is applicable to various plant tissues.

Ultra-pure: The obtained gDNA with high purity can be used directly in PCR, enzyme digestion, hybridization and other molecular biology experiments.

Important Notes Please read the notes before using this kit.

1. Avoid freezing and thawing of the sample, otherwise the DNA fragments extracted will be smaller and the amount of extraction will be decreased.
2. Buffer LP1 may appear to be yellow, but it will not affect the extraction efficiency.
3. If there's precipitation in Buffer LP1 or LP2, please incubate it in 37°C water bath and shake it before use.
4. All centrifugation steps should be carried out in a conventional table-top microcentrifuge at room temperature (15-25°C).

Protocol

Centrifugation Protocol

Please add ethanol (96%-100%) into Buffer LP3 and Buffer PW as indicated on the bottle before use.

1. Sample preparation:

   Add liquid nitrogen into 100 mg fresh plant tissues or 30 mg dry mass tissues and grind them sufficiently. Add 400 µl Buffer LP1 and 6 µl RNase A (10 mg/ml) into each grinded sample. Vortex for 1 min and incubate at room temperature for 10 min.

   Notes: To prevent moistening of plant tissues, you may pre-mix Buffer LP1 and RNase A based on the above ratio when the sample number is large.

2. Add 130 µl Buffer LP2, mix thoroughly and votex for 1 min.
3. Centrifuge for 5 min at 12,000 rpm(~13,400 × g).
4. Transfer the supernatant into N96 Filtration Plate (H) . Place N96 Filtration Plate (H) on N96 Well Plate and seal it with a plate cover. Centrifuge for 10 min at 3600 rpm(~2,130 × g), and transfer the filtrate to the N96 Wellp Plate.

   Notes: If there's plant tissues not thoroughly grinded or fragments which may block the holes, do not transfer them to N96 Filtration Plate (H) or else the holes will be blocked.

5. Add 1.5 times the supernant volume of Buffer LP3 to N96 Well Plate (for instance, add 750 µl Buffer LP3 **(check if ethanol was added before use)** into 500 µl of filtrate) and seal it with a plate cover. Mix thoroughly by votex for 15 sec (or mix them by pipette), flocculent precipitation may appear .
6. Add the solution and the flocculent precipitation obtained from the last step into N96 Plate CB3 (H) (put it on N96 Well Plate) and seal it with a plate cover. Centrifuge for 5 min at 3600 rpm (~2,130 × g), discard the flow-through and place N96 Plate CB3 (H) back to N96 Well Plate.
7. Add 600 µl Buffer PW to N96 Plate CB3 (H) **(check if ethanol was added before use)** and seal it with the plate cover. Centrifuge for 5 min at 3600 rpm (~2,130 × g), discard the flow-through and place N96 Plate CB3 (H) back to the N96 Well Plate.
8. Add 600 µl Buffer PW to N96 Plate CB3 (H) and seal it with a plate cover. Centrifuge for 5 min at 3600 rpm (~2,130 × g) and discard the flow-

through.

Notes: If the filtration plate membrane turns green, add 500 µl ethanol(96%-100%) to N96 Plate CB3 (H) and centrifuge for 5 min at 3600 rpm (~2,130 × g). Discard the flow-through and put N96 Plate CB3 (H) back to N96 Well Plate.

9. Centrifuge for 5 min at 3600 rpm(~2,130 × g) and discard the flow-through. Place N96 Plate CB3 (H) at room temperature for a few minutes to thoroughly dry out the residual ethanol.

   Notes: The purpose of this step is to remove the residual ethanol which can affect the following experiments such as enzyme digestion and PCR.

10. Transfer N96 Plate CB3 (H) to a new N96 Well Plate and add 50-200 µl Buffer TE to the middle of membrane. Incubate at room temperature for 2-5 min and centrifuge at 3600 rpm (~2,130 × g) for 8 min. Collect the solution in the N96 Well Plate.

    Notes: To increase the yield of the genomic DNA, the solution obtained from the centrifugation can be added again to the membrane, incubate at room temperature for 2 min and then centrifuge at 3600 rpm (~2,130 × g) for 8 min. The volume of the elution buffer should not be less than 50 µl, the recovery efficiency will be affected if the volume is too small. The pH value of the eluent has a great impact on the elution efficiency. If ddH_2O is used as the elution solution, make sure its pH value ranges from 7.0 to 8.5. The elution efficiency will be reduced if the pH value is smaller than 7.0. And the DNA product should be stored under -20°C to prevent DNA degradation.

Vacuum Protocol

The extraction steps 1-3 of the vacuum method are the same as the centrifugation process.

1. Connect the negative pressure vacuum device correctly and put N96 Filtration Plate (H) on the device, and put a new N96 Well Plate underneath it. Adjust the negative pressure to 40-70 kpa. Transfer the centrifuged supernatant solution to N96 Filtration Plate (H). Turn on the negative pressure device for 2 min to suction filtration.
2. Add Buffer LP3 of a volume 1.5 times to the filtrate into the filtrate on N96 Well Plate (for instance, add 750 µl of Buffer LP3 into 500 µl of filtrate) **(check whether ethanol has been added before using)** and mix well with pipette.
3. Take out N96 Filtration Plate (H) from the negative pressure device and put N96 Plate CB3 (H) on the device and place a waste liquid tank under it. Transfer all liquids and precipitations of the preceding steps on N96 Plate CB3 (H), turn on the device for 5 min of suction filtration.
4. Add 600 µl Buffer PW to N96 Plate CB3 (H) **(check whether ethanol has been added before using)**. Turn on the negative pressure device for 2 min to suction filtration.
5. Add 600 µl Buffer PW to N96 Plate CB3 (H). Turn on the negative pressure device for 5 min to suction filtration.
6. Turn off the negative pressure device and clean the waste liquid tank. Place N96 Plate CB3 (H) at room temperature for 3min.
7. Place the N96 Plate CB3 (H) on the negative pressure device and place a new N96 Well Plate under it. Add 50-200 µl Buffer TE to the middle of membrane and incubate for 2-5 min at room temperature. Turn on the negative pressure device for 3-5 min to suction filtration.