TIANGEN®

Hi-DNAsecure Plant Kit

Purification of genomic DNA from various plant tissues with high efficiency

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This product is for scientific research use only. Do not use in medicine, clinical treatment, food or cosmetics.

Hi-DNAsecure Plant Kit

Cat. no. 4992724/4992725

Kit Contents

Contents	4992724 (50 preps)	4992725 (200 preps)
Buffer FGA	40 ml	160 ml
Buffer LP2	10 ml	40 ml
Buffer LP3	21 ml	84 ml
Buffer PW	15 ml	50 ml
Buffer TB	15 ml	60 ml
RNase A (10 mg/ml)	300 μl	1.25 ml
Spin Columns CB3	50	200
Collection Tubes 2 ml	50	200
Handbook	1	1

Storage Conditions

Hi-DNAsecure Plant Kit should be stored dry at room temperature (15-30°C) and is stable for 12 months. For longer storage, the kit can be stored at 2-8°C. If precipitate has formed, please place the buffer at room temperature for a period of time before use, or warm in 37°C water bath for 10 min if necessary to dissolve the precipitate.

Introduction

This kit uses a centrifugal adsorption column that can specifically bind DNA and a unique lysis buffer system, which can efficiently extract genomic DNA from various plant tissues. The silicon matrix material used in the centrifugal adsorption column is a unique new material of TIANGEN, which can adsorb DNA efficiently and specifically and can remove impurity proteins to the maximum extent. The unique lysis buffer can efficiently lyse plant cells with high DNA yield, while protecting the integrity of DNA to the greatest extent. The extracted genomic DNA fragments are large, with high purity and stable and reliable quality.

The DNA purified by the kit is suitable for various conventional operations, including restriction enzyme digestion, PCR, library construction, Southern blot , etc.

Product Highlights

Easy and fast: Ultrapure genomic DNA can be obtained within 1 h.

Widely applicable: Applicable for various plant tissues.

Ultra purity and high efficiency: The DNA obtained with high efficiency has high purity and can be directly used in molecular biological experiments such as PCR, enzyme digestion, hybridization, etc.

Imporatant Notes **Please read this note before using this kit.**

1. Avoid repeated freezing and thawing of the sample, otherwise the extracted DNA fragments will be smaller and the yield will also decrease.
2. Buffer FGA may turn to yellow, but it does not affect the extraction effect.
3. If precipitate has formed in Buffer FGA or Buffer LP2, dissolve the buffer in 37°C water bath and used after mixing.
4. All centrifugation steps are performed using a bench-top centrifuge at room temperature.

Protocol

Ensure that 96%-100% ethanol has been added into Buffer LP3 and Buffer PW as indicated on the tag of the bottle before use.

1. Treatment of material

   Take 100 mg of fresh plant tissue or 20 mg of dry weight tissue, and grind thoroughly in liquid nitrogen. Add 400 μl of Buffer FGA and 6 μl RNase A (10 mg/ml), vortex for 1 min and place at room temperature for 10 min.

2. Add 130 μl of Buffer LP2, mix well by vortexing for 1 min.
3. Centrifuge at 12,000 rpm (~ 13,400 × g) for 5 min and transfer the supernatant to a new centrifuge tube.
4. Add 1.5 times the volume of Buffer LP3 (e.g. add 750 μl Buffer LP3 to 500 μl filtrate) **(Check whether 96-100% ethanol has been added before use)**, immediately and fully mix for 15 sec. Flocculant precipitation may occur at this moment.
5. Add the solution and flocculent precipitate obtained in the previous step to a Spin Column CB3 (Place the Spin Column CB3 in a collection tube), centrifuge at 12,000 rpm (~ 13,400 × g) for 30 sec, discard the flow through and put the Spin Column CB3 in the collection tube.
6. Add 600 μl of Buffer PW to the Spin Column CB3 **(Check whether 96-100% ethanol has been added before use)**. Centrifuge at 12,000 rpm (~ 13,400 × g) for 30 sec, discard the flow through, and place the Spin Column CB3 into the collection tube.

   Note: If the Spin Column membrane turns to green, add 500 μl of absolute ethanol to the Spin Column CB3, centrifuge at 12,000 rpm (~ 13,400 × g) for 30 sec, discard the flow through, and put the Spin Column CB3 into the collection tube.

7. Repeat step 6.
8. Put the Spin Column CB3 back into the collection tube, centrifuge at 12,000 rpm (~ 13,400 × g) for 2 min, and discard the flow through. Place the Spin Column CB3 at room temperature for several minutes to completely dry the residual Buffer PW.

   Note: The purpose of this step is to remove the residual Buffer PW in the Spin Column. The residual ethanol in Buffer PW will affect the subsequent enzyme reaction (enzyme digestion, PCR, etc.).

9. Transfer the Spin Column CB3 into a clean centrifuge tube, pipet 50-200 µl of Buffer TB directly into the middle of the adsorption membrane. Incubate at room temperature for 2-5 min, centrifuge at 12,000 rpm(~13,400 × g) for 2 min to elute.

   Note: In order to increase the yield of genomic DNA, add the solution obtained by centrifugation to Spin Column CB3 again, place at room temperature for 2 min and centrifuge at 12,000 rpm(~13,400 × g) for 2 min. The volume of the buffer should not be less than 50 µl. Too small a volume will affect the recovery efficiency. The pH value of the elution buffer has a great influence on the elution efficiency. If ddH$_2$O is used to elute, the pH value should be within the range of 7.0-8.5. The elution efficiency will be reduced if the pH value is lower than 7.0. The purified DNA should be kept at -20℃ to prevent DNA degradation.

Detection of DNA Concentration and Purity

The size of the obtained genomic DNA fragment is related to factors such as sample storage time and shearing force during operation. The concentration and purity of the purified DNA fragments can be detected by agarose gel electrophoresis and ultraviolet spectrophotometer.

DNA should have a significant absorption peak at OD_{260}, with OD_{260} value of 1 equivalent to about 50 µg/ml double stranded DNA and 40 µg/ml single stranded DNA.

The ratio of OD_{260}/OD_{280} should be 1.7-1.9. If ddH$_2$O is used instead of elution buffer, the ratio will be low because the pH value and the presence of ions will affect the light absorption value, but it does not mean the purity is low.