TIANprep N96 Magnetic Plasmid Kit

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Cat.no. 4992870

Kit Contents

Contents	4992870 (2 plates)
RNaseA (10 mg/ml)	600 μl
Buffer P1	60 ml
Buffer P2	60 ml
Buffer P3	80 ml
Buffer PW	50 ml
MagAttract Suspension G	3×1 ml
N96 Filtration Plate (H)	2
N96 Well Plate	4
Buffer EB	30 ml
Plate cover	10
Handbook	1

Storage

This kit can be stored dry at room temperature (15-25°C) for 12 months. 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. After RNase A is added to Buffer P1, it can be stored at 2-8°C for 6 months stably, and the separate packaged RNase A can be stored at room temperature for 12 months.

Introduction

This kit adopts magnetic beads with special separating function and unique buffer system, and can isolate and purify 2-20 μg high-quality plasmid DNA from 1-1.5 ml bacterial solution. The unique embedded magnetic beads have a strong affinity for plasmid DNA under certain conditions. When the conditions change, the magnetic beads release the adsorbed plasmid DNA, which can achieve the purpose of rapid separation and purification of plasmid DNA, and can remove protein and other impurities to the maximum extent, so as to ensure the purity of extracted plasmid DNA.

Plasmid DNA extracted by the kit can be used in various molecular biology experiments, such as enzyme digestion, sequencing, library screening, ligation and transformation, etc.

Important Notes Before Using

1. Please add RNaseA in Buffer P1 before use (the addition ratio is P1:RNaseA = 100:1), mixed evenly, and stored at 2-8°C.
2. Please add the corresponding volume of 96-100% ethanol to Buffer PW according to the label on the bottle before the using for the first time.
3. Before use, check whether Buffer P2 and Buffer P3 are turbid. If yes, incubate them in a 37°C water bath for a few minutes to restore clarification. Tighten the cap of Buffer P2 and Buffer P3 immediately after use.
4. All centrifugation steps shall be conducted at room temperature.
5. The amount of plasmid extracted is related to the culture concentration of bacteria, host bacteria, plasmid copy number and other factors.
6. The magnetic beads suspension should be fully mixed before use.

Protocol

Before use, please add 96-100% ethanol into Buffer PW according to the label on the bottle.

1. Bacteria collection step: Add 1.0-1.5 ml of shaken bacteria liquid to a new N96 Well Plate (or take the 96-well plate with shaken bacteria liquid), cover with Plate Cover, centrifuge at 3,600 rpm for 10 min to collect bacteria. Pour out the culture medium, and turn it upside down on an absorbent paper to remove the residual culture medium (if the concentration of bacteria liquid is low, the bacteria can be collected by repeating this step).
2. Add 250 µl of Buffer P1 to the collected bacterial culture **(please check whether RNaseA has been added)**, cover with a new Plate Cover, and completely suspend the bacteria by vortexing.
3. Remove the Plate Cover, add 250 µl Buffer P2 to each well of the N96 Well Plate, cover with a new Plate Cover, gently turn up and down for 6-8 times to fully lyse the thallus, and instantaneous centrifugation to make the solution on the Plate Cover fall back into the plate.

   Note: Gently mix well to prevent genomic DNA contamination. After mixing evenly, the bacterial solution shall be clear and viscous. The mixing time shall not exceed 5 min to avoid plasmid damage.
4. Remove the Plate Cover, add 350 µl Buffer P3 to each well of N96 Well Plate, cover with a new Plate Cover, immediately turn it up and down gently for 6-8 times to fully mix the solution, then centrifuge at 3,600 rpm for 10 min.

   Note: Buffer P3 shall be mixed immediately after addition to avoid local precipitation.
5. Place the N96 Filtration Plate in a new N96 Well Plate, transfer the supernatant from step 4 to the corresponding N96 Filtration Plate, and centrifuge at 3,600 rpm for 5 min.
6. Add 15 µl of MagAttract Suspension G to each well in the above N96 Well Plate, and slap to mix.
7. Leave the N96 Well Plate at room temperature for 5 min, slap to mix once during the incubation.
8. Place the N96 Well Plate on the magnetic stand and let it stand for 30 secs. Remove the supernatant when the magnetic beads are completely attached.

9. Remove the N96 Well Plate from the magnetic stand, add 600 µl of Buffer PW to each well **(please check whether 96-100% ethanol has been added)**, slap to mix.
10. Place the N96 Well Plate on the magnetic stand and let it stand for 30 secs. Remove the supernatant when the magnetic beads are completely attached.
11. Repeat steps 9 and 10 to remove the liquid as clean as possible.
12. Dry the N96 Well Plate on a magnetic stand at room temperature for 5-10 min.

    Note: Ethanol residue will inhibit the subsequent enzyme reaction, so make sure that the ethanol volatilizes completely when drying. Don't dry for too long to avoid difficulty in eluting DNA.
13. Remove the N96 Well Plate from the magnetic stand, add 50-100 µl Buffer EB, vortex to mix evenly, and let it stand at room temperature for 5-10 min, during which vortex to mix evenly once.
14. Place the N96 Well Plate on the magnetic stand and let it stand for 30 secs. When the magnetic beads are fully attached, carefully transfer the DNA solution to new tubes and store it under appropriate conditions.

    Note: The pH value of the eluent has a great influence on the elution efficiency, and the elution efficiency will be reduced if the pH value is lower than 7.0. DNA product shall be kept under -20°C to prevent DNA degradation.

Determination of Plasmid DNA Concentration and Purity

The concentration and purity of recovered plasmid DNA can be detected by agarose gel electrophoresis and ultraviolet spectrophotometer. The OD_{260} value of 1 corresponds to about 50 µg/ml of double-stranded DNA.

The ratio of OD_{260}/OD_{280} should be 1.7-1.9. If ddH_2O is used for elution instead of the elution buffer, the ratio will be low, but it does not mean low purity, because pH value and ion presence will affect light absorption value.