TIANGEN®

TIANprep N96 Plasmid Kit

For purification of molecular biology grade DNA

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TIANprep N96 Plasmid Kit

(N96 Plate)

Cat. no. 4992892/4992893

Kit Contents

Contents	4992892 4 plates	4992893 24 plates
Buffer BL	240 ml	3 ×500 ml
Buffer P1	125 ml	3×240 ml
Buffer P2	125 ml	3×240 ml
Buffer P3	160 ml	4×250 ml
Buffer PD	240 ml	3 ×500 ml
Buffer PW	3×50 ml	2 ×500 ml
Buffer TB	60 ml	240 ml
TIANRed	700 μl	4 ×1 ml
RNase A (10 mg/ml)	1.25 ml	6×1.25 ml
N96 Plate CP3(H)	4	24
N96 Filtration Plate(H)	4	24
N96 Well Plate	16	96
250 ml bottle	--	1
Plate Cover	16	96
Permeation	5	25
Handbook	1	1

Storage

TIANprep N96 Plasmid Kit can be stored dry at room temperature (15-30°C) for up to 15 months without showing any reduction in performance and quality. If any precipitate forms in the buffers, it should be dissolved by warming the buffers at room temperature or 37°C water bath for 10 min before use. RNase A (10 mg/ml) can be stored for 15 months at room temperature (15-30°C). After addition of RNase A, Buffer P1 is stable for 6 months at 2-8°C.

Introduction

TIANprep N96 Plasmid Kit uses spin plate which can specifically bind plasmid DNA. The silica membrane within the spin plate is made of our exclusive material which could specifically and efficiently bind plasmid DNA. Proteins and other contaminant compounds can be properly removed, and plasmid DNA that extracted by this kit is pure. No toxic reagent like phenol and chloroform included in this kit.

Plasmid DNA prepared by TIANprep N96 Plasmid Kit is suitable for a variety of molecular biological experiments including restriction enzyme digestion, sequencing, library screening, ligation, transformation, etc.

Important Notes Before Using

1. Germiculture in N96 plate: Add 1.0-1.3 ml culture medium with antibiotic into each well of N96 Well Plate, pick single colony into each well and cover the plate. Grow cell culture at 37°C for 20-24 h with shaking (220-280 rpm).
2. Add the provided RNase A solution to Buffer P1 before use **(please prepare in batches, add 1.25 ml RNase A concentration of 10 mg/ml per 125 ml P1, which may be used for 4-plate extraction reaction)**, mix, and store at 2-8°C.
3. Add 200 ml ethanol (96-100%) to 50 ml Buffer PW before using.
4. Check Buffer BL, P2 and P3 before use for salt precipitation. If necessary, dissolve the buffer by warming at 37°C water bath for several minutes. Immediately close the lid of Buffer P2 and P3 after use.
5. All centrifugation steps need to be carried out at room temperature (15-30°C).
6. The yield of plasmid DNA is related to cells concentration, bacteria strain and plasmid copy.
7. Using Buffer BL to treat spin plates could activate silica membrane and increase yield.
8. After treated with Buffer BL, use the Spin plate in the same day, otherwise lit may affect the purifying effect.

TIANRed user's guide

TIANRed is a color indicator which is used to ensure the experimental operation is correct. It has no negative effect on any downstream analysis and no harm to human body. TIANRed is an optional reagent.

TIANRed should be added into Buffer P1 before use in the ratio of 1:200, mix by reverting after the addition. Buffer P1 will turn red after the addition of TIANRed. If the reaction turns purple after the addition of P2, it indicates complete lysis. If the reaction turns yellow after the addition of Buffer P3, it indicates complete renatuation.

Protocol: Centrifugal protocol or vacuum protocol could be selected.

(I) Centrifugal protocol

1. Plate equilibration: Place N96 Plate CP3 into N96 Well Plate, and add 500 μl Buffer BL to each well of N96 plate CP3. Centrifuge for 3 min at 3,600 rpm (~2,130 × g). Discard the flow-through, and set the N96 plate CP3 back into the N96 Well Plate. **(Please use the plates in the same day)**
2. Bacteria harvest: Add 1.0-1.3 ml cultured bacterial cells in a N96 Well Plate (or directly culture the cells in N96 Well Plate), cover the plate and harvest cells by centrifuge at 3,600 rpm (~2,130 × g) for 10 min, then remove all traces of supernatant by inverting the open N96 Well Plate on filter paper until all medium has been drained. (This step could be repeated once to increase the amount of cells)
3. Resuspend pelleted bacterial cells in 250 μl Buffer P1 **(Ensure that RNase A has been added to Buffer P1)**. Cover the plate and mix by vortex.

   Note: Buffer P1 will turn red if TIANRed was applied.
4. Remove the plate cover and add 250 μl Buffer P2, cover the plate and mix thoroughly by inverting 6-8 times, centrifuge briefly to collect all the liquid.

   Note: Mix gently by inverting the plate. If TIANRed was applied, solution should turn purple after proper mix, or else part of the solution would still show red. Solution should become viscous and slightly clear after mix. Do not allow the lysis reaction to proceed for more than 5 min to protect plasmid DNA.

5. Remove the plate cover and add 350 μl Buffer P3, cover the plate with a new cover and mix immediately and thoroughly by inverting the plate 6-8 times.

   Note: To avoid localized precipitation, mix the solution thoroughly immediately after addition of Buffer P3. If TIANRed was applied, the reaction mix should turn yellow after mix.

6. Put a N96 Filtration Plate into a new N96 Well Plate, carefully transfer 750 μl solution from step 5 to the N96 Filtration Plate, centrifuge for 5 min at 3,600 rpm (~2,130 × g).

7. Carefully transfer all the flow-through from step 6 to the equilibrated N96 Plate CP3 **(place the N96 Plate CP3 into a N96 Well Plate).** Centrifuge for 5 min at 3,600 rpm (~2,130 × g), discard the flow-through and put the N96 Plate CP3 back into the N96 Well Plate.

8. Recommended: Wash the N96 Plate CP3 by adding 500 μl Buffer PD and centrifuging for 5 min at 3,600 rpm (~2,130 × g). Discard the flow-through and set the N96 Plate CP3 back into the N96 Well Plate.

   Note: This step is necessary to remove trace nuclease activity when using end A^+ strains such as the TG1, BL21, HB101 and JM series, which have high levels of nuclease activity that may degrade plasmid DNA, This step could be skipped if using end A^- strains (DH5α, TOP10).

9. Wash the N96 Plate CP3 by adding 700 μl Buffer PW **(ensure the ethanol (96%-100%) has been added to Buffer PW)** and centrifuging for 5 min at 3,600 rpm (~2,130 × g). Discard the flow-through and place the N96 Plate CP3 back into the N96 Well Plate.

10. Repeat Step 9.

11. Centrifuge the N96 Plate CP3 for an additional 10 min at 3,600 rpm (~2,130 × g) to remove residual wash buffer PW.

    Note: Residual ethanol from Buffer PW may inhibit subsequent enzymatic reactions (Enzyme digestion and PCR).

12. Place the N96 Plate CP3 in a clean N96 Well Plate. To elute DNA, add 80-100 μl Buffer TB or water (pH≥7.5) to the center of the each well of N96 Plate CP3, let stand for 5-6 min, and then centrifuge for 10 min at 3,600 rpm (~2,130 × g).

    Note: Normally 55 μl of DNA product could be eluted if 100 μl of elution buffer is used. More elution buffer could be used to increase the plasmid DNA yield (such as use 120 μl elution buffer). Besides,

the pH value of elution buffer has significant influence on eluting; if distilled water is used to elute DNA, the pH value should be within 7.0-8.5. Low pH value (pH<7) would reduce the efficiency of eluting. Extracted plasmid DNA should be stored at -20°C to avoid degradation.

(II) Vacuum protocol

1. Plate equilibration: Place N96 Plate CP3 into vacuum system, and add 500 μl Buffer BL to each well of N96 plate CP3. Turn on the vacuum system and evacuate the plate **(Please use the plates in the same day).**
2. Same as step 2-5 of (I) Centrifugal protocol.
3. After the step 5 of (I)Centrifugal protocol, place a N96 Filtration Plate and the equilibrated N96 Plate CP3 on the vacuum system, each well of the Filtration Plate need to correspond to each well of N96 Plate CP3.

   Note: Please follow the manual of the vacuum system to proceed this step.
4. Carefully transfer 750 μl solution from step 3 to the N96 Filtration Plate, and then evacuate the plate.
5. Recommended: Wash the N96 Plate CP3 by adding 500 μl Buffer PD and evacuate the plate.

   Note: This step is necessary to remove trace nuclease activity when using end A^+ strains such as the TG1, BL21, HB101 and JM series, which have high levels of nuclease activity that may degrade plasmid DNA. This step could be skipped if using end A^- strains (DH5α, TOP10).
6. Wash the N96 Plate CP3 by adding 700 μl Buffer PW and evacuate the plate.
7. Repeat Step 6.
8. Evacuate the plate in the maximum negative pressure for 10 min to remove residual wash buffer PW. If there is still liquid left after evacuation, use filter paper to remove them.
9. Place the N96 Plate CP3 into a clean N96 Well Plate. To elute DNA, add 80-100 μl Buffer TB or water (pH≥7.5) to the center of the each well of N96 Plate CP3, let stand for 5-6 min, and then centrifuge for 10 min at 3,600 rpm (~2,130 × g) to collect the plasmid DNA solution in the well plate.

Measurement of DNA concentration and purity

The concentration and purity of isolated plasmid DNA could be analyzed by agarose gel electrophoresis and UV spectrophotometry.

DNA has a significant peak at OD_{260}. An OD_{260} of 1 corresponds to a 50 μg/ml of dsDNA solution or a 40 μg/ml of ssDNA solution. $OD_{260}/_{280}$ ratio value should be within 1.7-1.9.