TIANGEN®

TIANprep Rapid N96 Plasmid Kit

www.tiangen.com/en

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

TIANprep Rapid N96 Plasmid Kit

(N96 Plate)

Cat. no. 4992868/4992869

Kit Contents

Contents	4992868 (4 plates)	4992869 (24 plates)
Buffer P1	125 ml	3×240 ml
Buffer P2	125 ml	3×240 ml
Buffer III	125 ml	3×240 ml
Buffer TB	60 ml	240 ml
TIANRed	700 ul	4×1 ml
RNase A (10 mg/ml)	1.25 ml	6×1.25 ml
N96 Filtration Plate (H)	4	24
N96 Well Plate	8	48
Plate Cover	26	150
Permeation	5	25
Handbook	1	1

Storage

It is the kit can be stored at room temperature (15-25°C) in a drying condition for 12 months. For longer storage, please store at 2-8°C. If the solution produces precipitation under the temperature of 2-8°C, place at room temperature for a period of time before use. If necessary, it may be placed in a 37°C water bath for 10 min to dissolve the precipitation. After RNase A is added to Buffer P1, it shall be stored at 2-8°C and can be stably stored for 12 months. The separately packed RNase A can be stored at room temperature for 12 months.

Introduction

TIANprep Rapid N96 rapid plasmid DNA extraction kit is suitable for rapid preparation of high-throughput plasmids, and may complete plasmid DNA extraction of 96 samples within 2-3 hours. This kit adopts the special N96 Filtration Plate (H) and Buffer III, which may quickly and efficiently extract high purity plasmid DNA.

The plasmid DNA purified by the kit is suitable for large-scale sequencing and conventional molecular biological operations, such as enzyme digestion, library screening, ligation, transformation, etc.

Important Notes **Please read the notes before using this kit.**

1. Culture method of 96 well plate bacteria: Add 1.0-1.3 ml of culture medium containing corresponding antibiotics to each well of 96 deep-well plates, then pick a single colony into each well to culture. Seal the plate with permeation to prevent contamination, and culture at 220-280 rpm 37°C.
2. RNase A shall be added to the Buffer P1 before use (please prepare in batches, add 1.25 ml RNase A per 125 ml P1, which may be used for 4-plate extraction reaction), mix well, and store at 2-8°C.
3. Before use, check whether Buffer P2 and Buffer III are turbid. If there is turbid phenomenon, incubate in a 37°C water bath for a few minutes to restore clarification. Cover Buffer P2 and Buffer III immediately after use.
4. All centrifugation steps shall be conducted at room temperature.
5. The yield of extracted plasmid is related to factors such as bacterial culture concentration and plasmid copy number.

Operation instructions of TIANRed

TIANRed is a color indicator to indicate the correctness of the whole operation, which does not affect any downstream experiments and is harmless to human body. TIANRed is an optional reagent, and customers may choose whether to add it or not according to their needs.

Usage method: Before use, add according to TIANRed:P1=1:200, and mix it upside down until it is completely uniform. The Buffer P1 added with TIANRed is red when in use; After Buffer P2 is added, the red color completely turns purple, indicating full lysis; after Buffer III is added again, the homogeneous state is yellow, indicating that neutralization and renaturation are sufficient.

Protocol: Centrifugal protocol or vacuum protocol could be selected.

(I) Centrifugal protocol

1. Add 1.0-1.3 ml of shaken bacteria solution to N96 Well Plate (or take N96 Well Plate with shaken bacteria solution), and cover with plate cover, then centrifuge at 3,600 rpm (~ 2,130 × g) for 10 min to collect bacteria. Discard the culture medium, and turn it upside down on absorbent paper to remove the residual culture medium. (If the thallus concentration is low, the bacteria may be collected again).
2. Add 250 μl of Buffer P1 to each well of the 96-well deep-well plate **(please check whether RNase A has been added first)**, and cover with plate cover, and completely suspend the bacteria pellet using a vortex.

   Notes: If Buffer P1 is added with TIANRed reagent, it is red at this time.
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 pellet, and instantaneous centrifugation to make the solution on the plate cover fall back into the plate.

   Notes: Mix gently and do not shake violently to avoid interrupting genomic DNA. If TIANRed reagent is used, the color will be purple when completely mixed, and some red remains will be found if lysis is not sufficient. After mixing evenly, the bacterial solution shall be clear and viscous, and the time shall not exceed 5 min to avoid plasmid damage.
4. Remove the plate cover, add 250 μl 4°C cooled Buffer III to each well of the N96 Well Plate, and cover with a new plate cover. Immediately gently turn it up and down for about 10 times, and fully mix it, then leave it at room temperature for 5 min, and instantaneously centrifuge the solution on the plate cover back into the plate.

   Notes: Buffer III shall be mixed immediately after addition to avoid local precipitation. If TIANRed reagent is used, the color will change from purple to yellow after being completely mixed.
5. *(An optional step) boil that N96 Well Plate in a boiling water bath for 5 min*.

   Notes: This treatment step is recommended when the host bacterium is end A^+ (TG1, JM series and its derivatives, HB101) to prevent the plasmid from being difficult to preserve for a long time due to nuclease contamination. If the host bacterium is end A^- (DH5α, TOP10), this step may be omitted. The boiling time shall not exceed 5 min, otherwise genome pollution is easy to occur.

6. *(An optional step) transfer the N96 Well Plate into an ice water bath for about 15 min and cool sufficiently to room temperature.*

   Notes: This step is essential if step 5 is applied. If step 5 is not applied, this step can be skipped. The heated N96 Well Plate shall be fully cooled to a low temperature by this step, otherwise the isopropanol precipitation step will be affected.

7. Remove the plate cover, and transfer the lysis buffer in Step 4 or Step 6 into a 96-well filter plate (the filter plate is stacked on a new sterile N96 Well Plate), and centrifuge at 3,600 rpm (~ 2,130 × g) for 5 min.

   Notes: After centrifugation, the water droplets on the surface of the N96 Well Plate are completely adsorbed with absorbent paper. If the absorbent paper does not completely absorb the water on the board surface, it may lead to the separation of the plate cover from the N96 Well Plate due to poor sealing after isopropanol is added in the next step.

8. Add 500 μl of isopropanol at room temperature to each well of the filtered clear solution, cover with a new plate cover, and mix it upside down for 5-6 times, then centrifuge at room temperature 3,600 rpm (~ 2,130×g) for 20 min, and carefully pour out the supernatant, and turn it upside down on absorbent paper.

   Notes: In order to prevent cross-contamination, the newly capped plate cover must be completely tightly attached to the N96 Well Plate; After the supernatant is poured out, the plasmid DNA is colorless and transparent, which is not easy to see.

9. Add 600 μl 4°C of 70% precooled ethanol to each well, and cover with a new plate cover, then rinse plasmid DNA upside down for 3-4 times. Centrifuge at 3,600 rpm (~ 2,130 × g) for 5 min, and carefully pour out the supernatant, then turn it upside down on absorbent paper, and air dry for 15- 20 min or vacuum dry for 10 min.

   Notes: If the drying is incomplete, the residue of ethanol will affect the subsequent enzyme reaction (enzyme digestion, PCR, etc.) experiments.

10. Add 50-100 μl Buffer TB into N96 Well Plate with a pipette, and cover with a new plate cover, and vortex for 1-2 min to help dissolve plasmid DNA.

    Notes: The optimal dissolution volume depends on the copy number of plasmid and the DNA concentration required for downstream experiments.

The pH value of the solution has a great influence on the long-term preservation of plasmids. If ddH_2O is used as the elution solution, make sure its pH value ranges from 7.0-8.5. The elution efficiency of DNA will be lowered if the pH value is smaller than 7.0; And the DNA product shall be kept under -20°C to prevent DNA degradation.

(II) Vacuum protocol

1. The following steps are the same as the operation methods of Steps 1-6 in (I) centrifugal method.
2. After the Step 6 of (I) centrifugal method, the plate cover is removed. Place a new sterile N96 Well Plate into the negative pressure vacuum device, and place the N96 Filtration Plate on the support of the negative pressure vacuum equipment. Each well of the N96 Filtration Plate shall be inserted into each well of the N96 Well Plate. The lysis buffer in the N96 Well Plate is pipetted into the N96 Filtration Plate, and the negative pressure vacuum is adjusted to drain the solution.

   Notes: After drying, the water droplets on the surface of the N96 Well Plate are completely sucked with absorbent paper. If the absorbent paper does not completely absorb the water on the board surface, it may lead to the separation of the plate cover from the N96 Well Plate due to poor sealing after isopropanol is added in the next step.
3. Add 500 μl of isopropanol at room temperature to each well of the filtered clear solution, and cover with a new plate cover, and mix it upside down for 5-6 times, then centrifuge at room temperature 3,600 rpm (~ 2,130 × g) for 20 min, and carefully discard the supernatant, and turn it upside down on absorbent paper to absorb the water in the plate.

   Notes: In order to prevent cross-contamination, the newly capped plate cover must be completely tightly attached to the N96 Well Plate; After the supernatant is poured out, the plasmid DNA is colorless and transparent, which is not easy to see.
4. Add 600 μl 4°C of 70% precooled ethanol to each well, and cover with a new plate cover, then rinse plasmid DNA upside down for 3-4 times. Centrifuge at 3,600 rpm (~ 2,130 × g) for 5 min, and carefully pour out the supernatant, then turn it upside down on absorbent paper, and air dry for 15-20 min or vacuum dry for 10 min.

   Notes: If the drying is incomplete, the residue of ethanol will affect the subsequent enzyme reaction (enzyme digestion, PCR, etc.) experiments.

5. Add 50-100 µl Buffer TB into N96 Well Plate with a pipette, and cover with a new plate cover, and vortex for 1-2 min to help dissolve plasmid DNA.

   Notes: The optimal dissolution volume depends on the copy number of plasmid and the DNA concentration required for downstream experiments.

   The pH value of the solution has a great influence on the long-term preservation of plasmids. If ddH_2O is used as the elution solution, make sure its pH value ranges from 7.0-8.5. The elution efficiency of DNA will be lowered if the pH value is smaller than 7.0; And the DNA product shall be kept under -20°C to prevent DNA degradation.