TIANpure Mini Plasmid Kit

For purification of molecular biology grade DNA

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TIANpure Mini Plasmid Kit

(Spin Column)

Cat. no. 4992424

Kit Contents

Contents	4992424 50 preps
RNase A (10 mg/ml)	150 µl
Buffer BL	30 ml
Buffer P1	15 ml
Buffer P2	15 ml
Buffer P3	20 ml
Buffer PD	30 ml
Buffer PW	15 ml
Buffer TB	15 ml
Filtration Columns CS	50
Spin Columns CP3	50
Collection Tubes 2 ml	100
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Storage

TIANpure Mini 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 37°C 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

TIANpure Mini Plasmid Kit is developed based on alkaline lysis technology followed by adsorption of DNA onto silica membrane in the presence of high salt. Filtration Columns CS is provided to remove proteins and organic compounds exist in cells. This protocol is designed for purification of plasmid DNA from 1-5 ml overnight cultures of *E.coli* in LB (Luria-Bertani) medium. The yield and quality is depended also on strain type, cell lysis, plasmid copy number, plasmid stability, used antibiotics, etc.

Plasmid DNA prepared by TIANpure Mini Plasmid Kit is suitable for a variety of routine applications including restriction enzyme digestion, sequencing, library screening, ligation and trans-formation, in vitro translation, and transfection of robust cells.

Yield

Plasmid Type	Bacterial Cells Volume	Plasmid Yield	Plasmid
Low Copy	1-5 ml	3-12 μg	pBR322, pACYC, pSC101, SuperCos, pWE15
High Copy	1-5 ml	6-30 μg	pTZ, pUC, pBS, pGM-T

Important Notes Before starting

1. Add the provided RNase A solution to Buffer P1 before use **(use 1 vial RNase A per bottle Buffer P1)**, mix, and store at 2-8°C.
2. Check Buffer BL, P2 and P3 before use for salt precipitation. If necessary, dissolve the buffer by warming at 37°C for several minutes.
3. Avoid direct contact of Buffer P2 and P3, immediately close the lid after use.
4. All centrifugation steps are carried out at 12,000 rpm (~13,400 × g) in table-top microcentrifuge at room temperature (15-30°C).
5. The amount of extracted plasmid is related to cells concentration and plasmid copy.
6. Using Buffer BL to treat spin columns could activate silica membrane at maximum degree and higher yield.
7. After treated with Buffer BL, use the Spin Column soon, otherwise long-term placement may affect the purifying effect.

Protocol

Add ethanol (96-100%) to Buffer PW before use, check bottle tag for the adding volume.

1. Column equilibration: Place a Spin Column CP3 in a clean collection tube, and add 500 µl Buffer BL to CP3. Centrifuge for 1 min at 12,000 rpm (~13,400×g) in a table-top microcentrifuge. Discard the flow-through, and set the Spin Column CP3 back into the collection tube.
2. Harvest 1-5 ml bacterial cells in a microcentrifuge tube by centrifugation at 12,000 rpm (~13,400×g) in a conventional, table-top microcentrifuge for 1 min at room temperature (15-30°C), then remove all traces of supernatant by gently inverting the open centrifuge tube until all medium has been drained.
3. Resuspend pelleted bacterial cells in 250 µl Buffer P1.

   Note: Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet.
4. Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 6-8 times.

   Note: Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min. If the lysate is still not clear, please reduce bacterial pellet.
5. Add 350 µl Buffer P3 and mix immediately and thoroughly by inverting the tube 6-8 times. The solution should become cloudy.

   Note: To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer P3.
6. Centrifuge for 10 min at 12,000 rpm (~13,400×g) in a table-top microcentrifuge. A compact white pellet will form. Carefully transfer the supernatant from step 6 to the Filtration Columns CS (place the CS in a collection tube) by decanting or pipetting. Centrifuge for 2 min at 12,000 rpm (~13,400×g).

   Note: Avoid pipetting out the precipitate.
7. Transfer the flow-through in the collection tube to the Spin Column CP3 by decanting or pipetting. Centrifuge for 1 min at 12,000 rpm (~13,400×g). Discard the flow-through and set the Spin Column CP3 back into the Collection Tube.

8. Wash the Spin Column CP3 by adding 500 µl Buffer PD and centrifuging for 1 min at 12,000 rpm (~13,400×g). Discard the flow-through and set the CP3 back into the Collection Tube.
9. Wash the Spin Column CP3 by adding 600 µl Buffer PW (ensure the ethanol (96%-100%) has been added to Buffer PW) and centrifuging for 30-60 sec at 12,000 rpm (~13,400×g). Discard the flow-through and set the CP3 back into the Collection Tube.
10. Wash Spin Column CP3 by adding 600 µl Buffer PW and centrifuging for 1 min at 12,000 rpm (~13,400×g).
11. Discard the flow-through, and centrifuge for an additional 2 min at 12,000 rpm (~13,400×g) to remove residual wash Buffer PW.

    Note: Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. We suggest open CP3 lid and let it stand at room temperature for a while. Residual ethanol from Buffer PW may inhibit subsequent enzymatic reactions.
12. Place the Spin Column CP3 in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50-100 µl Buffer TB or water (pH7.0-8.5) to the center of the Spin Column CP3, let it stand for 2 min, and then centrifuge for 2 min at 12,000 rpm (~13,400×g).

    Note: If the volume of eluted buffer is less than 50 µl, it may affect recovery efficiency. The pH value of eluted buffer will have some influence in eluting; Buffer TB or distilled water (pH7.0-8.5) is suggested to elute plasmid DNA. Repeat step 12 to increase plasmid recovery efficiency.

Extraction of Low Copy or Large Plasmid (> 10 kb)

For low copy plasmids and plasmids larger than 10 kb, the amount of bacteria should be increased. It is recommended to use 5-10 ml overnight culture, and the volume of Buffer P1, P2 and P3 should be increased in proportion. Buffer EB should be preheated in 65-70°C water bath, and the incubation time for adsorption and elution can be appropriately prolonged to increase the extraction efficiency. The other steps are the same as the above protocol.