TIANpure Midi Plasmid Kit

For purification of molecular biology grade DNA

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TIANpure Midi Plasmid Kit

(Spin Column)

Cat. no. 4992421

Kit Contents

Contents	4992421 50 preps
RNase A (10 mg/ml)	300 µl
Buffer BL	30 ml
Buffer P1	30 ml
Buffer P2	30 ml
Buffer P3	40 ml
Buffer PD	30 ml
Buffer PW	15 ml
Buffer TB	15 ml
Filtration Columns CS	50
Spin Columns CP4	50
Collection Tubes 2 ml	100
Handbook	1

Storage

TIANpure Midi 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 to 37°C before use. RNase A (10 mg/ml) can be stored for 15 months at room temperature (15-30°C). After adding RNase A, Buffer P1 is should be stored at 2-8°C and is stable for 6 months.

Introduction

TIANpure Midi Plasmid Kit is based on alkaline lysis technology followed by adsorption of DNA onto silica membrane in the presence of high salt. Plasmid DNA purified with TIANpure Midi Plasmid Kit is immediately ready for use. Phenol extraction and ethanol precipitation are not required. High-quality plasmid DNA is eluted in a small volume of Tris Buffer or deionized water. This protocol is designed for purification of plasmid DNA from 5-15 ml overnight culture of *E.coli* in LB (Luria-Bertani) medium.

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

Yield

Plasmid Type	Bacterial Cells Volume	Plasmid Yield	Plasmid
Low Copy	5-15 ml	5-25 μg	pBR322, pACYC, pSC101, SuperCos, pWE15
High Copy	5-15 ml	15-70 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.
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 centrifuge at room temperature.
5. The amount of extracted plasmid is related to cells concentration and plasmid copy. If working with low copy vectors or large plasmid (>10 kb), it may be beneficial to increase culture volume and to increase Buffer P1, P2, and P3 in proportion. Warm the Buffer TB to 65-70°C before use. **Prolong adsorption and elution time properly to increase extraction efficiency.**

6. Use 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, since 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 CP4 in a clean collection tube, and add 500 µl Buffer BL to CP4. Centrifuge for 1 min at 12,000 rpm (~13,400 × g) in a table-top centrifuge. Discard the flow-through, and set the Spin Column CP4 back into the collection tube. **(Please use freshly treated spin column).**
2. Harvest 5-15 ml bacterial cells by centrifugation at 12,000 rpm (~13,400 × g) in a conventional, table-top centrifuge for 1 min at room temperature (15-30°C), then remove all traces of supernatant.

   Note: For large volume of bacterial cells, please harvest to one tube by several centrifugation step. Too much bacterial cells will lead to incomplete lysis and further reduce plasmid yield.
3. Re-suspend the bacterial pellet in 500 µl Buffer P1 **(Ensure that RNase A has been added to Buffer P1)**. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

   Note: No cell clumps should be visible after re-suspension of the pellet, otherwise incomplete lysis will lower yield and purity.
4. Add 500 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 lysate 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 700 µl Buffer P3 and mix immediately and thoroughly by inverting the tube 6-8 times. The lysate should become cloudy. Centrifuge for 10 min at 12,000 rpm (~13,400 × g) in a table-top centrifuge. A compact white pellet will form at this step.

Note: Mix the solution thoroughly immediately after addition of Buffer P3 to avoid localized precipitation. If there is still white precipitation in the supernatant, please centrifuge again.

6. Carefully transfer the supernatant from step 5 to Filtration Column CS **(place the CS in a collection tube).** Centrifuge for 2 min at 12,000 rpm (~13,400×g) in a table-top centrifuge. Carefully transfer the flow-through to Spin Column CP4 **(place CP4 into a collection tube)(residual supernatant left in the filtration column indicates too much impurity in supernatant obtained in step 5, please centrifuge for longer time; if precipitate appears in the collection tube bottom, try to pipet the supernatant as much as possible).**
7. Centrifuge for 1 min at 12,000 rpm (~13,400×g). Discard the flow-through and set the Spin Column CP4 back into the Collection Tube.
8. Wash the Spin Column CP4 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 CP4 back into the Collection Tube.
9. Wash the Spin Column CP4 by adding 600 µl Buffer PW **(Ensure that ethanol (96%-100%)has been added)** and centrifuging for 1 min at 12,000 rpm (~13,400 ×g). Discard the flow-through and set the CP4 back into the Collection Tube.

   Note: Leave the CP4 at room temperature for 2-5 min after adding Buffer PW will help to remove impurities.

10. Repeat step 9.
11. Centrifuge for 2 min at 12,000 rpm (~13,400×g) to remove residual wash buffer PW.

    Note: Residual ethanol from Buffer PW may inhibit subsequent enzymatic reactions. We suggest opening Spin Column CP4 lid and stand at room temperature for a while to completely dry the membrane.

12. Place the Spin Column CP4 in a clean 1.5 ml centrifuge tube, add 100-300 µl Buffer TB to the center of the Spin Column CP4, stand for 2 min, and centrifuge for 1 min at 12,000 rpm (~13,400×g) to elute.

    Note: If the volume of elution buffer is less than 100 µl, it may affect recovery efficiency. DNA product should be stored at -20°C to avoid degradation. The pH value of elution buffer will have big influence in eluting. If using distilled water, pH should be controlled at 7.0-8.5,

below 7.0 will affect elution efficiency. To achieve higher elution efficiency, transfer the obtained product to spin column again, stand at room temperature for 2 min, then centrifuge for 2 min at 12,000 rpm (~13,400×g) to elute again.

Determination of DNA Concentration and Purity

The recovered DNA fragments can be detected by agarose gel electrophoresis and ultraviolet spectrophotometer. DNA should have a significant absorption peak at OD_{260}. OD_{260} value of 1 is equivalent to about 50 μg/ml double stranded DNA and 40 μg/ml single stranded DNA. The OD_{260}/ OD_{280} ratio should be 1.7-1.9. If ddH_2O is used for the elution instead of the elution buffer, the ratio will be lower, because the pH value and the presence of ions will affect the light absorption value, but it does not mean the purity is low.