TIANprep Midi Plasmid Kit

For purification of molecular biology grade DNA

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TIANprep Midi Plasmid Kit

(Spin Column)

Cat.no. 4992425

Kit Contents

Contents	4992425 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 EB	15 ml
Spin Columns CP4	50
Collection Tubes 2 ml	50
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Storage

TIANprep Midi Plasmid Kit can be stored 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 adding RNase A, Buffer P1 is should be stored at 2-8°C and is stable for 6 months.

Introduction

TIANprep 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 TIANprep 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 TIANprep 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 to 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 Please read before use.

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, immediately close the lid after use.
4. All centrifugation steps are carried out at 12,000 rpm (~13,400× g) in table-tap microcentrifuge at room temperature (15-30°C).
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. Using Buffer BL to treat spin columns could activate silica membrane at maximum degree and the higher yield.
7. After treated with Buffer BL, use the Spin Column CP4 soon, since long-term placement may affect the purifying effect.

8. Buffer PD can effectively remove residual protein. This step is essential when working with endA+ strains such as TG1, BL21, HB101, ET1256, JM101, etc., to ensure that plasmid DNA is not degraded.

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 microcentrifuge. 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 in a microcentrifuge tube by centrifugation at 12,000 rpm (~13,400 × g) 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 vortex or pipetting up and down until no cell clumps remain.
   Note: No cell clumps should be visible after resuspension 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.
   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 the Spin Column CP4 (**Capacity: 750-800 µl. place the CP4 into a collection tube**), please note not to touch precipitate. 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.

7. *(optional) 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.*

   Note: This step is necessary to remove trace nuclease activity when using endA+ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content.

8. Wash the Spin Column CP4 by adding 600 µl Buffer PW **(ensure the ethanol (96-100%) has been added to Buffer PW)** 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 Spin Column CP4 by adding 600 µl Buffer PW and centrifuging for 1 min at 12,000 rpm (~13,400 × g).
10. 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 ethanol from Buffer PW may inhibit subsequent enzymatic reactions. We suggest open CP4 lid and stay at room temperature for a while to get rid of residual ethanol.

11. Place the Spin Column CP4 into a clean 1.5 ml microcentrifuge tube. To elute DNA, add 100-300 µl Buffer EB or ddH_2O (pH 7.0-8.5) to the center of the Spin Column CP4, and incubate for 2 min, and centrifuge for 1 min at 12,000 rpm (~13,400 × g).

    Note: Repeat step 11 to increase plasmid recovery efficiency. If the volume of eluted buffer is less than 100 µl, it may affect recovery efficiency. The pH value of eluted buffer will have some influence in eluting. Buffer EB or distilled ddH_2O (pH 7.0-8.5) is suggested to elute plasmid DNA. For long-term storage of DNA, eluting in Buffer EB and storing at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis.