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TIANprep Mini Plasmid Kit

For purification of molecular biology grade DNA

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TIANprep Mini Plasmid Kit

(Spin Column)

Cat. no. 4992423/4992420

Kit Contents

Contents	4992423 50 preps	4992420 200 preps
RNase A (10 mg/ml)	150 µl	600 µl
Buffer BL	30 ml	120 ml
Buffer P1	15 ml	60 ml
Buffer P2	15 ml	60 ml
Buffer P3	20 ml	80 ml
Buffer PD	30 ml	120 ml
Buffer PW	15 ml	50 ml
Buffer EB	15 ml	30 ml
Spin Columns CP3	50	200
Collection Tubes 2 ml	50	200
Handbook	1	1

Storage

TIANprep Mini Plasmid Kit can be stored dry at room temperature (15-25°C) for up to 12 months without showing any reduction in performance and quality. For longer storage, this kit can be stored at 2-8°C. If any precipitate forms in the buffers after storage at 2-8°C, it should be dissolved by warming the buffers at 37°C before use. RNase A (10 mg/ml) can be stored for 12 months at room temperature (15-25°C). After addition of RNase A, Buffer P1 is stable for 12 months at 2-8°C.

Introduction

TIANprep Mini 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 Mini 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 1-5 ml overnight cultures of *E. coli* in LB (Luria-Bertani) medium.

Plasmid DNA prepared by TIANprep 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 a table-top microcentrifuge at room temperature (15-25°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.
8. Buffer PD can effectively remove residual protein. This step is essential when working with endA+ strains such as TG1, BL21, HB101, ET12567, JM series, 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 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 put the Spin Column CP3 back into the collection tube. **(Please use freshly treated spin column).**
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 (15-25°C), then remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained (For large volume of bacterial cells, please harvest to one tube by several centrifugation step.)
3. Re-suspend the bacterial pellet in 250 µl Buffer P1 **(Ensure that RNase A has been added).** 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 250 µl Buffer P2 and mix gently and 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 gently by inverting the tube 6-8 times. The solution should become cloudy. Centrifuge for 10 min at 12,000 rpm (~13,400 × g) in a table-top microcentrifuge.

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

6. Transfer the supernatant from step 5 to the Spin Column CP3 **(place CP3 in a collection tube)** by decanting or pipetting. Centrifuge for 30-60 sec at 12,000 rpm (~13,400 × g). Discard the flow-through and set the Spin Column CP3 back into the Collection Tube.

7. *(Optional) Wash the Spin Column CP3 by adding 500 µl Buffer PD and centrifuge for 30-60 sec at 12,000 rpm (~13,400 × g). Discard the flow-through and put Spin Column CP3 back to the collection tube.*

   This step is recommended 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 CP3 by adding 600 µl Buffer PW **(ensure that ethanol (96%-100%) has been added)** and centrifuge for 30-60 sec at 12,000 rpm (~13,400 × g). Discard the flow-through, and put the Spin Colum CP3 back into the Collection Tube.

9. Repeat Step 8.

10. Centrifuge for an additional 2 min at 12,000 rpm (~13,400 × g) to remove residual Buffer PW.

    Note: Residual ethanol from Buffer PW may inhibit subsequent enzymatic reactions. We suggest open CP3 lid and stay at room temperature for a while to get rid of residual ethanol.

11. Place the Spin Column CP3 in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50-100 µl Buffer EB to the center of the Spin Column CP3, incubate for 2 min, and 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 EB or distilled water (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. Repeat step 11 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.