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TIANprep Mini Plasmid Kit Protocol V.2

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Abstract

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 1-5ml 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.

Guidelines

Applicable to TIANprep Mini Plasmid Kit manufactured by TIANGEN.

Materials

RNase A, Buffer BL, Buffer P1, Buffer P2, Buffer P3, Buffer PD, Buffer PW, Buffer EB, Spin Columns CP3, Collection Tubes 2ml

Troubleshooting



Safety warnings

⚠ Avoid direct contact with Buffer P2 and P3.

Before start

Buffer P1 must be activated with RNase A solution before use (one vial per one bottle) and store at 2-8 degrees celsius after activation.



Preparation of Bacterial Cells

16m

- 1 Centrifugation of  1-5 mL of bacterial cells in a microcentrifuge tube at  12000 rpm, Room temperature, 00:01:00 , table-top microcentrifuge

1m



Note

Amount of bacterial cells to be added can be divided into two parts rather than being added at once. For example,  4 mL of bacterial cells can be divided to  2 mL and centrifuged before discarding the flow-through discarded and add another  2 mL and centrifuged again to maximize the yield.

- 2 Direct drainage of supernatant by opening and inverting the tube.

Expected result

Bacteria pellet should be present in the microcentrifuge tube.

- 3 Complete resuspension of the bacteria pellet in  250 μ L of **Buffer P1**.



Note

- Buffer P1 must be pre-activated with **RNase A**.
- No cell clumps should be visible after resuspension. Vortex and pipette can be used to help homogenize the mixture.

- 4 Add  250 μ L of **Buffer P2** (lysis buffer) and mix by inverting the tube 6-8 times.

5m

Safety information



Avoid direct contact with Buffer P2

**Note**

- **DO NOT** vortex or violently mix the reaction.
- **DO NOT** allow the lysis reaction to continue for more than

- 5 Add  350 μL of **Buffer P3** and mix immediately by inverting the tube 6-8 times to neutralize Buffer P2.

**Safety information**

Avoid direct contact with Buffer P3

Expected result

There should be no white precipitation left in the supernatant. If there is, the mixture should be centrifuged again.

- 6 Centrifuge at  12000 rpm, Room temperature, 00:10:00 , table-top microcentrifuge

10m

**Preparation of Spin Column CP3**

1m

- 7 Place Spin Column CP3 in a clean collection tube.

- 8 Add  500 μL of **Buffer BL** to CP3.



- 9 Centrifuge at  12000 rpm, Room temperature, 00:01:00 , table-top microcentrifuge

1m



- 10 Discard the flow-through and put the Spin Column CP3 back into the collection tube.

Plasmid Extraction

9m



11 Transfer of supernatant from **Step 6** to Spin Column CP3 with collection tube attached.



12 Centrifuge at  12000 rpm, Room temperature, 00:01:00 , table-top microcentrifuge

1m



13 Discard the flow-through, retain Spin Column CP3 with collection tube attached.

14 **OPTIONAL:** Wash the Spin Column CP3 by adding  500 μL of **Buffer PD** and centrifuge at  12000 rpm, Room temperature, 00:01:00 , table-top microcentrifuge and discard the flow-through.

1m



Note

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.

15 Wash the column with Buffer PW

15.1 Add  600 μL of **Buffer PW**



Note

Buffer PW must be pre-treated with 96-100% ethanol.

15.2 Centrifuge at  12000 rpm, Room temperature, 00:01:00 , table-top microcentrifuge
.=

1m



15.3 Discard the flow-through, retain Spin Column CP3 with collection tube attached.

16 Repeat **Step 15**.



17 Centrifuge at  12000 rpm, Room temperature, 00:02:00 , table-top microcentrifuge to remove residual Buffer PW.

2m



18 Air dry the column at room temperature for a while to allow residual ethanol from Buffer PW to evaporate.

Note

Residual ethanol from Buffer PW may inhibit subsequent enzymatic reactions.

19 Place Spin Column CP3 in a clean 1.5 ml microcentrifuge tube. Discard the collection tube.

20 Add  50-100 μ L of **Buffer EB** to the center of the Spin Column CP3.



21 Incubate for  00:02:00

2m



22 Centrifuge at  12000 rpm, Room temperature, 00:02:00 , table-top microcentrifuge

2m



Protocol references

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