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In Use

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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 readyfor 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 avariety 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.

PROTOCOL integer ID: 82947

MATERIALS

Keywords: plasmid DNA

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

SAFETY WARNINGS

•

Avoid direct contact with Buffer P2 and P3.

BEFORE START INSTRUCTIONS

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

Centrifiguation of 🔼 1-5 mL of bacterial cells in a microcentrifuge tube at

1m



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

Note

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

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

Expected result

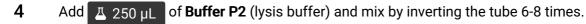
Bacteria pellet should be present in the microcentrifuge tube.

3 Complete resuspension of the bacteria pellet in \square 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.





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

Add Δ 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.

Centrifuge at



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

10m

5m

Preparation of Spin Column CP3

1m

7 Place Spin Column CP3 in a clean collection tube.

8 Add \mathbb{Z} 500 μL of **Buffer BL** to CP3.



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.

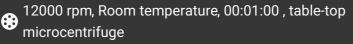


Centrifuge at Centrifuge at 12000 rpm, Room temperature, 00:01:00 , table-top microcentrifuge

1m

- Discard the flow-through, retain Spin Column CP3 with collection tube attached.
- OPTIONAL: Wash the Spin Column CP3 by adding $\boxed{2}$ 500 μ L of Buffer PD and centrifuge at





and discard the flow-

through.

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 \angle 600 μ L 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

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

Repeat Step 15.

Centrifuge at

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

to remove



residual Buffer PW.

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



21 Incubate for 00:02:00



Centrifuge at

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

2m