TIANprep Mini Plasmid Kit Protocol V.2

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DISCLAIMER

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

Keywords: plasmid DNA

MATERIALS

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

1 Centrifuguation 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 µL 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 µL 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

1m

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