TOOLS Endotoxin Free Plasmid Mini Kit

For purification of endotoxin free (<0.1 EU/μg) plasmid DNA

Contents

Introduction

The TOOLS Endotoxin Free Plasmid Mini Kit add a columns-free endotoxin removal step into the plasmid purification procedure that is based on the use of alkaline lysis technology, followed by adsorption of DNA onto a silica membrane in the presence of a high level of salt. Plasmid DNA purified with this product 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 up to 30 μg of plasmid DNA (<12 kb) from 3~4 mL of overnight cultures of E. coli in Luria Bertani medium. Plasmid DNA prepared by this kit is suitable for a variety of routine applications, including restriction enzyme digestion, sequencing, library screening, ligation and transformation, in vitro translation, and transfection of robust cells.

Important Note

1. Preparing Buffer P1: Add 0.5 mL of Buffer P1 to the provided RNase A and mix well, then add the mixture (Buffer P1 and RNase A) back into the residual Buffer P1. Mix everything well and store at 2ºC~8°C.
2. Add 200 mL absolute ethanol to Buffer W2 before use (check bottle label for volume).
3. Check Buffer P1, Buffer P2, and Buffer P3 before use for salt precipitation. Dissolve the buffers every time there is any precipitate by warming to 37 °C. Do not shake Buffer P2 vigorously.
4. Close the bottle containing Buffers P2 and P3 immediately after use to avoid acidification due to contact with CO_2 in the air.
5. All centrifugation steps are conducted at 12,000 rpm approximately 13,400 × g in a microcentrifuge at room temperature.

Kit contents

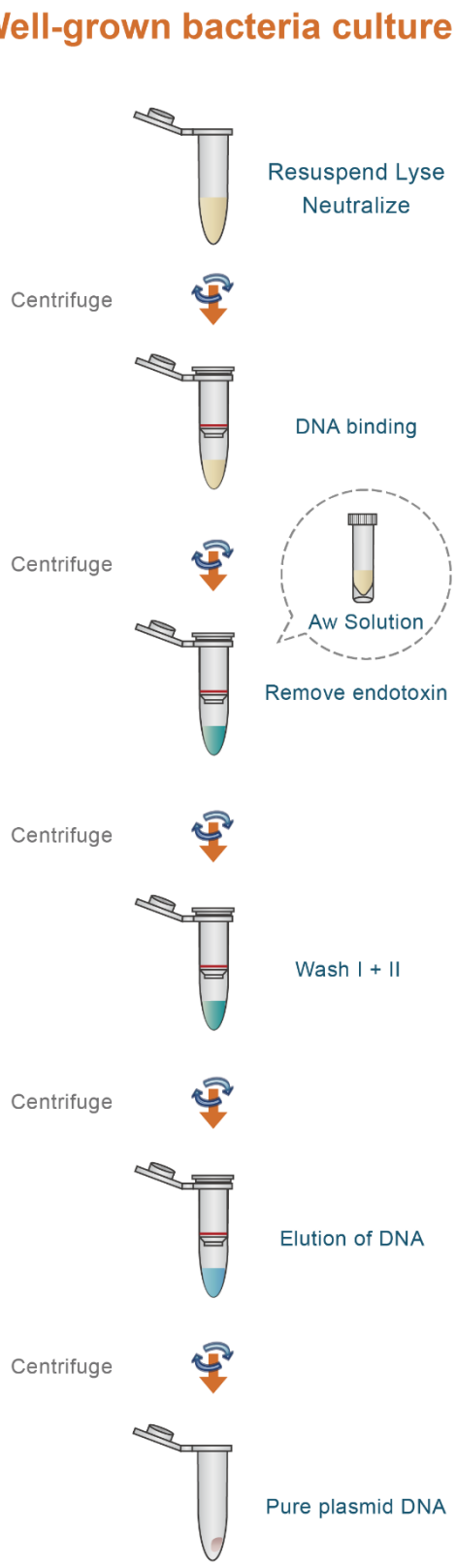
Component	ST-EF01 (300 preps)
RNase A (powder)	7.5 mg
Buffer P1	75 mL
Buffer P2	75 mL
Buffer P3	105 mL
Buffer BD	35 mL
Buffer AW	70 mL
Buffer W1	135 mL
Buffer W2*(Add Ethanol), 2 bottles	50 mL
Buffer EB	35 mL
Spin Columns CP3	300 tubes
Collection Tubes (2 mL)	300 tubes

*Add 200 mL absolute ethanol into Buffer W2 before initial use.

Storage

- Store RNase A at -20°C upon receiving the kit.
- Buffer P1 (added RNase A) should be stored at 2ºC~8°C.
- The other components in TOOLS Endotoxin Free Plasmid Mini Kit can be stored dry at room temperature (15ºC~25°C) for up to 12 months without showing any reduction in performance or quality.

Workflow


Well-grown bacteria culture
Resuspend Lyse
Neutralize
Centrifuge
DNA binding
Centrifuge
Aw Solution
Remove endotoxin
Centrifuge
Wash I + II
Centrifuge
Elution of DNA
Centrifuge
Pure plasmid DNA

Protocol

1. Harvest **3~4 mL** of bacterial cells in a microcentrifuge tube by centrifugation at 12,000 rpm (~13,400 × g) in conventional table-top microcentrifuge for 1 min at room temperature (15°C-25°C), and then remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained.
2. Resuspend pelleted bacterial cells in **200 µL** of **Buffer P1**.
3. Add **200 µL** of **Buffer P2** and mix thoroughly by inverting the tube 10 times.

Note: Mix gently by inverting the tube. Do not vortex, as this will result in the 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.

4. Add **300 µL** of **Buffer P3** and mix immediately and thoroughly by inverting the tube 10 times. The solution should become cloudy.

Note: To avoid localized precipitation, mix the solution thoroughly and immediately after the addition of Buffer P3. Do not vortex, as this will result in the shearing of genomic DNA.

5. Centrifuge for 10 min at 12,000 rpm (~13,400 × g) in a table-top microcentrifuge. A compact white pellet will form.
6. Mix the supernatants from step 5 with **100 µL Buffer BD** thoroughly by inverting the tube 10 times. Add the mixture of supernatants and Buffer BD to spin column CP3 (place the CP3 in a collection tube) by decanting or pipetting. Centrifuge for 1 min at 12,000 rpm (~13,400 × g). Discard the flow-through.
7. Wash spin column CP3 by adding **200 µl** of **Buffer AW** and centrifuging for 1 min at 12,000 rpm (~13,400 × g) Discard the flow through.
8. Wash spin column CP3 by adding **400 µl** of **Buffer W1** and centrifuging for 1 min at 12,000 rpm (~13,400 × g) Discard the flow through.
9. Wash spin column CP3 by adding **700 µl** of **Buffer W2** (ensure the absolute ethanol has been added to Buffer W2) and centrifuging for 1 min at 12,000 rpm (~13,400 × g) Discard the flow through.
10. Repeat **step 9** again.
11. Centrifuge for an additional 3 min at 12,000 rpm (~13,400 × g) to remove residual Buffer W2.

Note: Residual Buffer W2 will not be completely removed unless the flow through is discarded before this additional centrifugation. We suggest opening the CP3 lid and letting the samples stand at room temperature for a while. Residual ethanol from Buffer W2 may also inhibit subsequent enzymatic reactions.)

12. Place spin column CP3 in a clean 1.5 mL microcentrifuge tube. To elute DNA, add 50~100 µL of **Buffer EB** or water (pH 7.0-8.5) to the center of each spin column CP3, let the samples stand for 2~5 min, and then centrifuge for 2 min at 12,000 rpm (~13,400 × g). Store the collected DNA solution at 4ºC or 20°C.

Note: The pH of the elution buffer may affect the efficiency of the extraction. Please ensure that the pH is within the range of 7.0-8.5 if using water as the elution buffer. For low copy or >10 kb plasmid, 5-10 mL of the bacterial cells is required to scale up the Buffer P1, Buffer P2, and Buffer P3. The Buffer EB should be preheated to 60ºC-70°C to extend the time of DNA binding.)