TOOLS Plasmid Mini Kit

For purification of molecular biology-grade DNA

Contents

Introduction

The TOOLS Plasmid Mini Kit 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 1–4 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 Notes

1. Preparing P1 buffer: Add 0.5 mL of Buffer P1 to the provided RNase A and mix well, then add the mixture (P1 buffer and RNase A) back into the residual P1 buffer. Mix everything well and store at 2ºC–8°C.
2. Add 200 mL ethanol (96%–100%) to buffer PW before use (check bottle label for volume).
3. Check buffers P1, P2, and 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 table-top microcentrifuge at room temperature.

Yields

Plasmid size	Bacterial Cells Volume	Plasmid Yield	Time consumed
< 12kb	1-4 mL	20-30 μg	25 minutes

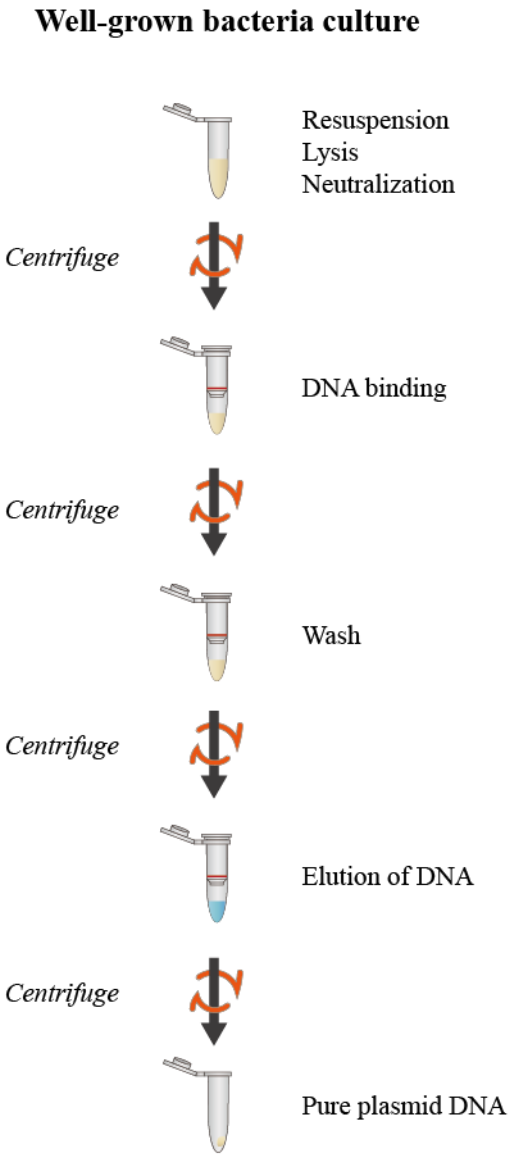
Kit Contents

Contents	TT-A03-3 (300 preps)
RNase A (powder)	7.5 mg
Buffer P1	75 mL
Buffer P2	75 mL
Buffer P3	105 mL
Buffer PD	135 mL
Buffer PW	50 mL
Buffer EB	35 mL
Spin Columns CP3	300
Collection Tubes（2 mL）	300

Storage

The TOOLS 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. If any precipitate forms in the buffers after storage at 2ºC–8°C, it should be dissolved by warming the buffers to 37°C before use. Store RNase A at –20°C upon receiving the kit.

Workflow


Well-grown bacteria culture
Resuspension
Lysis
Neutralization
Centrifuge
DNA binding
Centrifuge
Wash
Centrifuge
Elution of DNA
Centrifuge
Pure plasmid DNA

Protocol

1. Harvest 1–4 mL of bacterial cells in a microcentrifuge tube by centrifugation at 12,000 rpm (approximately 13,400×*g*) in a conventional table-top microcentrifuge for 1 min at room temperature (15ºC–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 (approximately 13,400×*g*) in a table-top microcentrifuge. A compact white pellet will form.
6. Add the supernatants from step 5 to spin column CP3 (place the CP3 in a collection tube) by decanting or pipetting. Centrifuge for 1 min at 12,000 rpm (approximately 13,400×*g*). Discard the flow-through.
7. Recommended: Wash spin column CP3 by adding 400 µl of buffer PD and centrifuging for 1 min at 12,000 rpm (approximately 13,400×*g*). Discard the flow-through.
8. Wash spin column CP3 by adding 700 µl of buffer PW (ensure the ethanol [96%–100%] has been added to buffer PW) and centrifuging for 1 min at 12,000 rpm (approximately 13,400×*g*). Discard the flow-through.
9. Centrifuge for an additional 3 min at 12,000 rpm (approximately 13,400×*g*) to remove residual wash buffer PW.

Note: Residual wash buffer 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 PW may also inhibit subsequent enzymatic reactions.

10. 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 (approximately 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 P1, P2, and P3 buffers. The EB buffer should be preheated to 60ºC–70°C to extend the time of DNA binding.