TOOLS Quick Plasmid Maxi Kit

For quick purification of ultrapure plasmid DNA with high yields

Contents

Introduction

The TOOLS Quick Plasmid Maxi kit is based on the alkaline lysis principle and utilizes the finalizer to prevent the loss of precious samples. It is recommended to use 100 mL of bacterial cells for high-copy vectors, yielding 800~1,000 μg of plasmid DNA. The entire process, from bacterial cells to obtaining pure plasmid DNA, takes less than 1 hour. Specifically, plasmid DNA adheres to the finalizer in the presence of a high salt concentration, while Buffer PB4 and Filtration Column CS are employed to effectively eliminate endotoxins and protein impurities. This product is suitable for a wide range of routine applications, including restriction enzyme digestion, sequencing, library screening, ligation, transformation, as well as in vitro translation and the transfection of robust cells.

Important Note

1. Add the provided RNase A solution to Buffer PB1, mix, and then store it at 2°C~8°C.
2. Add absolute ethanol to Buffer PBW before use (check the bottle label for the volume). For example, add 165 mL of absolute ethanol to 70 mL of Buffer PBW.
3. Before using Buffers PB2 and PB4 in salt precipitation, check for any precipitate. If there is any, redissolve it by warming the buffer to 37°C. Do not shake Buffer PB2 vigorously.
4. Prevent direct contact between Buffer PB2 and Buffer PB4. Immediately close the bottles containing Buffer PB2 and Buffer PB4 after use to prevent the acidification of both buffers (due to contact with CO2 in the air).
5. When withdrawing the plunger from the filter, do so slowly to avoid loosening the membrane.
6. Remove the finalizer from the syringe before pulling out the plunger from the syringe.

Kit contents

Component	ST-BA01 (10 preps)
RNase A (powder)	500 µL
Buffer PB1	100 mL
Buffer PB2	100 mL
Buffer PB4	100 mL
Buffer PBW	70 mL
Buffer TB	30 mL
Filtration Columns CS	10
Finalizers	10
Syringe (50 mL)	10
Syringe (1 mL)	10

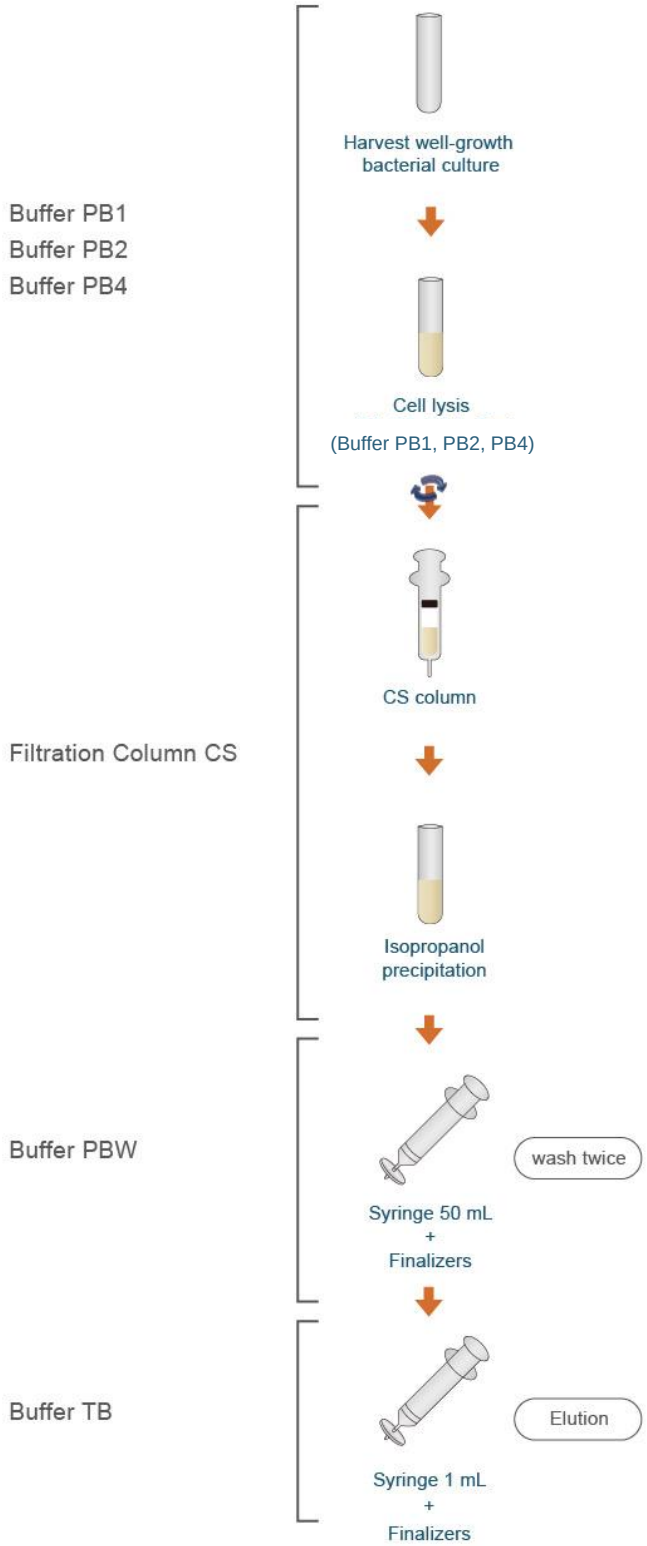
*Add 165 mL absolute ethanol into Buffer PBW before initial use.

*Absolute ethanol, isopropanol and 5M NaCl are not supplied with the kit.

Storage

The TOOLS Quick Plasmid Maxi kit can be stored dry at room temperature (15°C~25°C) for up to 12 months with no detriment to performance and quality. RNase A (100 mg/mL) can be stored for one year at room temperature (15°C~25°C). After RNase A is added, Buffer PB1 is stable for 6 months at 2°C~8°

Work Flow


Harvest well-growth bacterial culture
Buffer PB1
Buffer PB2
Buffer PB4
Cell lysis
(Buffer PB1, PB2, PB4)
CS column
Filtration Column CS
Isopropanol precipitation
Buffer PBW
wash twice
Syringe 50 mL
+
Finalizers
Buffer TB
Elution
Syringe 1 mL
+
Finalizers

Protocol

1. Harvest **100 mL** of bacterial culture cells by centrifuging at 8,000 rpm (≈ 8,228 × g) for 3 minutes at room temperature (15°C~25°C). Remove all traces of supernatant by inverting the open centrifuge tube until the medium has completely drained.

2. Resuspend the pelleted bacterial cells in **8 mL** of **Buffer PB1**.

Note: Ensure that RNase A has been added to Buffer PB1. There should be no visible cell clumps after resuspending the pellet.

3. Add **8 mL** of **Buffer PB2** and mix thoroughly by gently inverting the tube six to eight times. Incubate at room temperature for 5 minutes.

Note: Mix by gently inverting the tube; do not vortex, as this may shear 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 minutes.

4. Add **8 mL** of Buffer **PB4** and mix it immediately and thoroughly by gently inverting the tube six to eight times until the entire solution becomes cloudy. Incubate at room temperature for **10 minutes**. During this incubation period, a white, fluffy material will form.

Note: To prevent localized precipitation, ensure thorough mixing of the lysate immediately after adding Buffer PB4 by gently inverting the tube; do not vortex. Importantly, incubate at room temperature for the full 10 minutes; skipping this step will result in lower plasmid DNA yield.

5. Centrifuge for 10 minutes at 8,000 rpm (≈ 8,228 × g). The white material will precipitate to the bottom of the centrifuge tube and form a white pellet.

6. Transfer the supernatant into a **Filtration Column CS**. Gently insert the plunger into the Filtration Column CS and filter the cell lysate into a new 50-mL tube (not supplied in the kit).

Note: If the white material fails to precipitate to the bottom in step 5, the lysate was not mixed thoroughly in step 5. In this case, avoid transferring large clumps into Filtration Column CS to prevent clogging of the filtration membrane. The presence of small fragments does not affect the application of Filtration Column CS.

7. Add **0.5 times** the volume (≈ 12 mL) of **isopropanol** and **0.25 times** the volume (≈ 6 mL) of **5M NaCl** to the cleared lysate. Seal the received tube and mix thoroughly.

8. Remove the plunger from the 50 mL syringe and attach a finalizer to the opening. Transfer the solution entirely to the 50 mL syringe with the finalizer attached. Reinsert the plunger and slowly press the solution vertically, drop by drop. Discard the flow-through.

9. Remove the finalizer from the 50 mL syringe, pull out the plunger from the 50 mL syringe, and reattach the finalizer to the 50 mL syringe opening. Add **10 mL** of **Buffer PBW** to the syringe, reinsert the plunger, and slowly press the Buffer PBW vertically, drop by drop. Discard the flow-through.

10. **Repeat step 9**.

11. Remove the finalizer from the 50 mL syringe, pull out the plunger from the 50 mL syringe, and reattach the finalizer to the 50 mL syringe opening. Add **6 mL** of **absolute ethanol** to the syringe, reinsert the plunger, and slowly press the absolute ethanol, drop by drop. Discard the flow-through.

12. Remove the finalizer from the 50 mL syringe, pull out the plunger from the 50 mL syringe, and reattach the finalizer to the 50 mL syringe opening. Place the lens tissue under the tip of the finalizer to absorb any residual ethanol and **press air** through the finalizer as forcefully as possible. Repeat this step at least 8 times until no more residual ethanol leaks from the finalizer.

13. Remove the finalizer from the 50 mL syringe, pull out the plunger from the 1 mL syringe, and attach the finalizer to the 1 mL syringe opening. Prepare a new collection tube to elute the plasmid DNA, add **1,000 µL** of **Buffer TB**, reinsert the plunger, and gently press down vertically to facilitate the collection of plasmid DNA. Repeating this step 1~2 times can enhance the plasmid DNA yield.

Note: If the eluted buffer volume is less than 400 µL, recovery efficiency may be affected. The pH of the eluted buffer partially affects elution. It is recommended to use Buffer TB or distilled water (pH: 7.0-8.5) for plasmid DNA elution. For long-term DNA storage, elution in Buffer TB and storage at -20°C is recommended, as storing in water may subject the DNA to acid hydrolysis.