# SiMax™ Plasmid DNA Miniprep Kit

# User's Instruction

**Cat No.: CSA-50**

## Description

The SiMax™ Plasmid DNA Miniprep Kit is designed for the rapid purification of plasmid DNA. In a high-salt buffer, DNA is bound to the SiMax™ membrane in a spin column. Following a wash step, DNA is eluted in low-salt buffer or water without the need for alcohol precipitation or desalting. 5~15 μg of high-copy number plasmid DNA can be isolated from 3 ml of bacterial culture in 30 minutes. This kit removes proteins, RNAs, and other low molecular weight impurities. The purified high-quality plasmid DNA is ready for routine molecular biology applications such as restriction enzyme digestion, PCR, automated sequencing, manual sequencing, and transformation.

## Kit Contents

| | 50 preps |
|---|---|
| 1. Resuspension Solution (Solution I) | 7 ml |
| 2. Lysis Solution (Solution II) | 10 ml |
| 3. Neutralization Solution (Solution III) | 20 ml |
| 4. Binding buffer | 25 ml |
| 5. Miniprep spin columns with 2 ml collection tubes | 50 |

**Notes:** Solution I is recommended to be stored at 4℃. Please warm it to room temperature before use. Other reagents can be stored at room temperature. When room temperature is below 25℃, some components in the solution or buffer may be crystallized or salted out. Please warm it until all are dissolved before use.

## Protocol

1. Pellet 3~5 ml of an overnight bacterial culture by centrifugation at 13,000 rpm for 30 seconds and decant the supernatant completely.

   **Note:** Complete removal of excess liquid is very important.

2. Resuspend the cell pellet in 100 μl Solution I by vortexing or pipetting.

**Note:** It is essential to thoroughly resuspend the cells.

3. Add 150 µl Solution II and mix gently by inverting the tube about ten times. Let the tube stand for 1~3 minutes until the cell suspension clears.

4. Add 150 µl Solution III and mix gently by inverting the tube about ten times. Centrifuge the bacterial lysate at 13,000 rpm for 8~10 minutes.

5. Transfer the supernatant to a 1.5 ml microtube. Add 0.4 ml Binding buffer to the tube. Mix thoroughly by inverting for approximately 1 minute.

6. Transfer the mixture into a Miniprep spin column with a 2 ml Collection tube. Wait for at least 3 minutes, and then centrifuge at 13,000 rpm for 30 seconds and discard the flow-through.

7. Add 600 µl of 80% isopropanol (or 80% ethanol) to the Spin column. Centrifuge at 13,000 rpm for 1 minute and discard the flow-through.

8. Repeat Step 7 one or two times to remove the residual isopropanol or ethanol.

9. Place the Spin column into a new 1.5 ml microtube. Open the lid for 2-3 minutes to let the ethanol volatilize completely. Add 50 µl TE buffer (50 µl ultrapure water instead for sequencing) into the center part of the SiMax™ membrane in the spin column. Incubate at room temperature for 3~5 minutes and then elute the plasmid DNA by centrifugation at 13,000 rpm for 1 minute.

   **Note:** Repeat this step once if more DNA is required.

10. Determine the quality of the purified plasmid DNA on 1% agarose gel stained with GoodView™ or EB. Store the plasmid DNA at 4 ℃ for immediate use or at -20℃ for future use.

**Optional:** Add 0.5 µl RNase A solution (10 mg/ml) and incubate at 37℃ for 30 minutes if there is any contamination of RNA. This step does not interfere with downstream applications.

## Additional Information

- If the amount of bacterial culture is much more than recommended above, the amount of Solution I, II, and III should be increased proportionally. Please contact us if any help is needed.

- Contamination of trace amount of RNA does not interfere with downstream applications such as restriction enzyme digestion, transformation, and sequencing.