SiMax™ Genomic DNA Extraction

User's Instruction

Cat No.: CSC-50

Description

The SiMax™ Genomic DNA Extraction Kit is designed for rapid, small scale isolation of genomic DNA from a wide range of samples. In a high-salt buffer, DNA is selectively bound to the SiMax™ membrane in a spin column. Following a wash step, DNA is eluted in low-salt buffer or water without alcohol precipitation or desalting. Although the protocol provided in this kit is for the isolation of genomic DNA from whole blood, the kit can also be used for extracting DNA from many other samples. Please refer to the supplementary protocol. DNA isolated with this kit is suitable for a variety of applications, including amplification, restriction enzyme digestion and membrane hybridizations (e.g. Southern and dot/slot blots).

Kit Contents

	50 preps
1. GN binding buffer	50 ml
2. Washing buffer	50 ml
3. Miniprep spin column with 2 ml collection tubes	50

Notes before use

When the room temperature is below 25℃, the GN binding buffer may be crystallized or salted out. Please warm it until all components are dissolved before use, and always keep GN binding buffer above 25℃ during the experiment.

Protocol

1. Centrifuge 0.5-1 ml whole blood at 13,000 rpm for 2 minutes and discard supernatant without disturbing the visible pellet.
2. Add 100 µl of TE buffer to the pellet and resuspend it by vortexing. Add 1 ml GN binding buffer and gently mix the mixture by inversion.
3. Transfer the above mixture to a Miniprep spin column with a 2 ml Collection tube. Let it stand for at least 3 minutes. Centrifuge at 13,000 rpm for 30 seconds and discard the flow-through.

4. Transfer the remaining mixture to the Spin column and repeat the step 3.

5. Add 0.5 ml of Washing buffer to the Spin column and centrifugate at 13,000 rpm for 30 seconds. Repeat this step one or two times to remove impurities as much as possible.

6. Centrifuge at 13,000 rpm for an additional 1 minute to remove the residual Washing buffer.

7. Place the spin column into a 1.5 ml microtube. Let the tube lid open for 2-3 minutes to volatilize Washing buffer completely.

8. Add 100 μl of TE buffer into the center part of the SiMax™ membrane in the spin column and incubate at room temperature for 3-5 minutes. Centrifuge at 13,000 rpm for 1 minute to elute DNA.

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

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

Supplementary protocol

The kit can be used to extract genomic DNA not only from whole blood, but also from bacterial cultures, cultured animal cells, animal tissues and plant cells.

Extraction of genomic DNA from bacterial cultures

A. **gram-negative bacteria** Pellet bacterial cells from 1.5 ml overnight culture (about 5×10^6 cells) by centrifugation at 13,000 rpm for 30 seconds. Resuspend the cells in 0.5 ml PBS buffer or TE buffer. Add 1 ml of GN binding buffer to the suspension and mix it thoroughly. Then follow the steps for extracting DNA from whole blood given above.

B. **gram-positive bacteria** Pellet bacterial cells from 1.5 ml overnight culture (about 5×10^6 cells) by centrifugation at 13,000 rpm for 30 seconds. Resuspend the bacterial cells in 400 μl of 50 mM EDTA solution. Add 100 μl of 10 mg/ml lysozyme to the suspension and incubate at 37℃ for 30-60 minutes, mix them gently. Add 1 ml of GN binding buffer to the mixture and mix it thoroughly. Then follow the steps for extracting DNA from whole blood given above.

Extraction of genomic DNA from animal tissues or cultured animal cells

A. **Animal tissues:** Weigh 20-50 mg animal tissues. Homogenize the tissue thoroughly in 1 ml of GN binding buffer; or grind the tissue to a fine powder with liquid nitrogen in a pre-cooled mortar and pestle. Transfer the ground tissue to a 1.5 ml microfuge tube. Add 1 ml of GN binding buffer and mix it thoroughly. Then follow the steps for extracting DNA from whole blood given above.

B. **Animal cultured cells:** Pellet 1.5 ml of cultured cells (about 5×10^6 cells) by centrifugation at 13,000 rpm for 30 seconds. Resuspend the cells in 500 µl PBS buffer or TE buffer. Add 1 ml of GN binding buffer to the suspension and mix it thoroughly. Then follow the steps for extracting DNA from whole blood given above.

Extraction of genomic DNA from plant cells

Weigh 50-100 mg plant tissues. Homogenize the plant tissue thoroughly in 0.5 ml of CTAB extraction buffer with a homogenizer; or grind the plant tissue to a fine powder with liquid nitrogen in a pre-cooled mortar and pestle, and transfer the ground tissue to 0.5 ml of CTAB extraction buffer pre-warmed to 65℃. Incubate at 65℃ for 15-20 minutes. Add 1 ml of GN binding buffer to the mixture and mix it thoroughly. Then follow the steps for extracting DNA from whole blood given above.

Preparation of buffers

- **TE buffer: 100 ml**

  Mix 1 ml of 1 mol/L Tris-HCl (pH7.6) and 0.2 ml of 0.5 mol/L EDTA (pH8.0) together. Add ultrapure water too 100ml. The solution can be autoclaved and stored at room temperature.

- **PBS buffer (phosphate-buffered saline): 100 ml**

  Dissolve 0.8g NaCl, 0.02g KCl, 0.144g Na2HPO4 and 0.024g KH2PO4 in 80 ml ultrapure water. Adjust the pH to 7.4 with HCl. Add ultrapure water to 100ml. The solution can be autoclaved and stored at room temperature.

- **CTAB extraction buffer: 100 ml**

  Mix 10 ml of 1 mol/L Tris-HCl (pH7.6), 14 ml of 5 mol/L NaCl and 4.0 ml of 0.5 mol/L EDTA (pH8.0) together. Dissolve 1 g DTT and 1 g CTAB in the solution. Then add ultrapure water to 100 ml. The solution can be autoclaved and stored at room temperature.