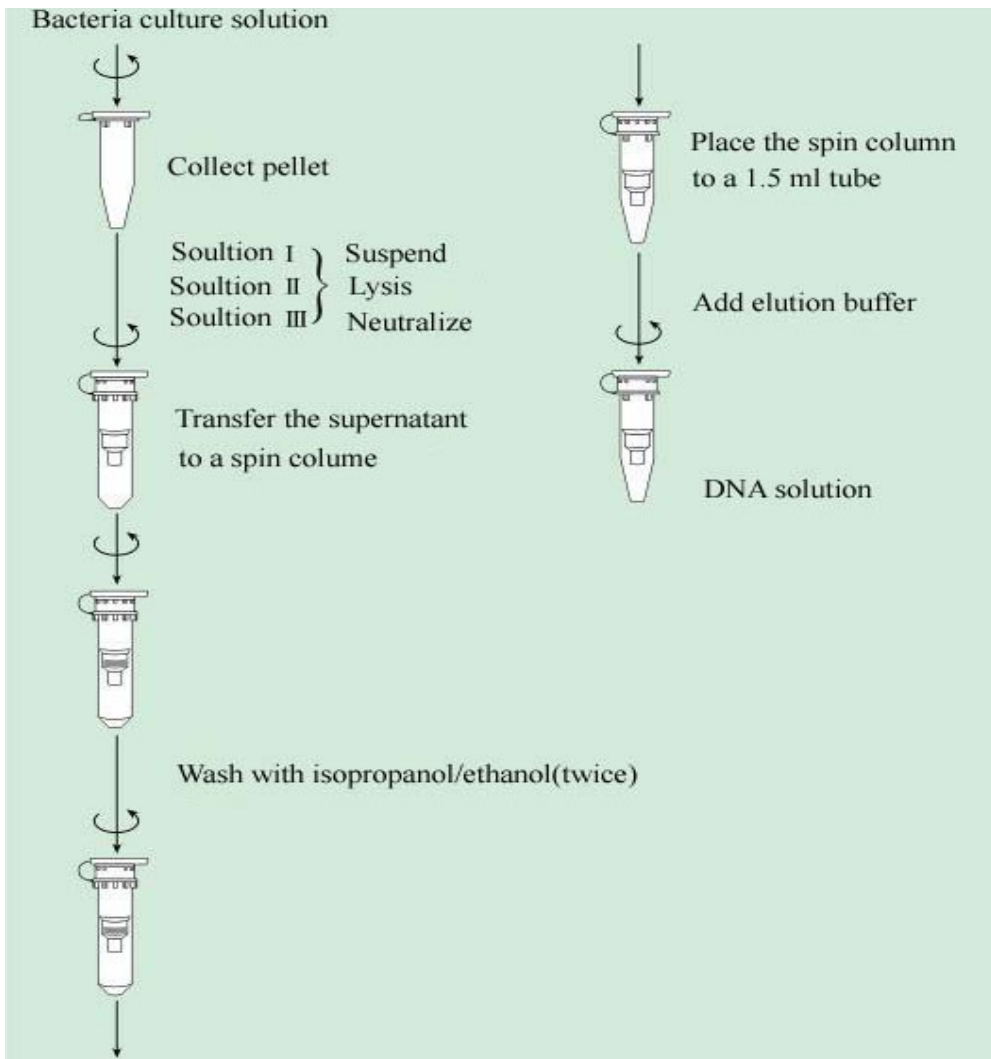


## **Nucleic Acids Isolation & Purification**

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## SiMax™ Plasmid DNA Miniprep

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, the DNA is eluted in low-salt buffer or water without alcohol precipitation or desalting. 5~15 µg of a high-copy number plasmid DNA can be isolated from 3 ml of bacterial culture in 30 minutes. This kit removes proteins, RNA, and low molecular weight impurities. The high-quality plasmid DNA can be used for restriction enzyme digestion, PCR, automated sequencing, manual sequencing, and transformation.



# SiMax™ Plasmid DNA Miniprep Kit User's Instruction

## 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

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

**Notes:** Solution I is recommended to store at 4°C. Please warm it to room temperature before use. Other reagents can be stored at room temperature. When room temperature is below 25°C, 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  $\mu$ l Solution I by vortexing or pipetting.

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

3. Add 150  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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. Add 50  $\mu$ l TE buffer (50  $\mu$ l ultrapure water instead for sequencing) into the center part of the SiMax<sup>™</sup> 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 GoldView<sup>™</sup> or EB. Store the plasmid DNA at 4 °C for immediate use or at -20 °C for future use.

**Optional:** Add 0.5  $\mu$ l RNase A solution (10 mg/ml) and incubate at 37°C for 30 minutes if there is any contamination of RNA. This step does not interfere

with downstream applications.

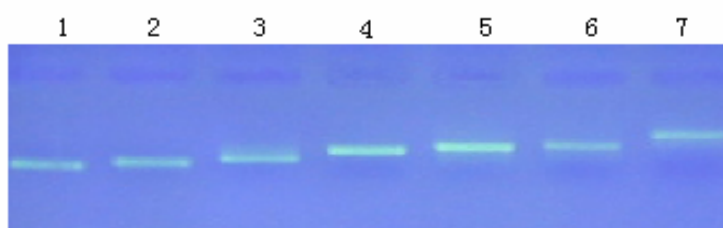
### **Additional Information**

- ◆ 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.

## SiMax™ PCR Products/Agarose Gel Purification

The SiMax™ PCR Products/Agarose Gel Purification Kit is designed for the rapid purification of PCR products, or for the efficient extraction of DNA fragments from agarose gel. 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 alcohol precipitation or desalting. This kit removes DNA polymerase, dNTPs, and salts. DNA fragments of 50 bp to 20 kb can be cleaned up within 20 minutes.

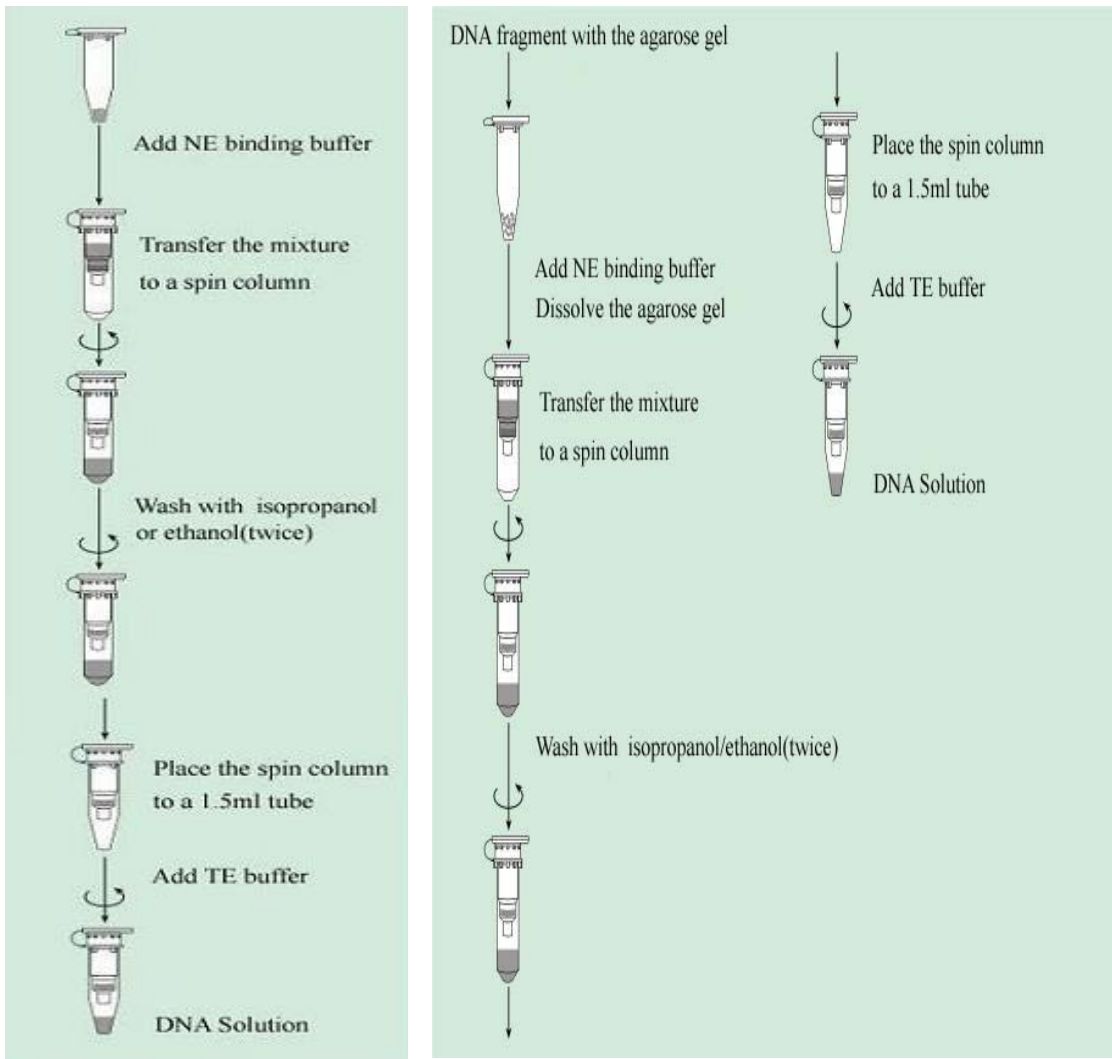
Known amount (2 µg) of seven DNA fragments was extracted from the agarose gel using SiMax™ PCR Products/Agarose Gel Purification Kit. 1/10 amount of each recovered fragment was analyzed by agarose gel electrophoresis. Recovery rate was up to 80%.



DNA fragment extracted from agarose gel.

### 1% agarose gel stained with GoldView™ (TAE buffer)

1. 200bp fragment recovered from gel
2. 300bp fragment recovered from gel
3. 400bp fragment recovered from gel
4. 500bp fragment recovered from gel
5. 750bp fragment recovered from gel
6. 900bp fragment recovered from gel
7. 1,000bp fragment recovered from gel



Purification of DNA amplified in PCR reactions  
 Extraction of DNA fragment from agarose gel

# SiMax™ PCR Products/Agarose Gel Purification User's Instruction

## Description

The SiMax™ PCR Products/Agarose Gel Purification Kit is designed for the rapid purification of PCR products, or for the efficient extraction of DNA fragments from agarose gels. 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 alcohol precipitation or desalting. This kit removes DNA polymerase, dNTPs, and salts. DNA fragments of 50 bp to 20 kb can be cleaned up within 20 minutes.

## Kit Contents

1. NE binding buffer	<u>50 preps</u> 50 ml
2. Miniprep spin columns with 2 ml collection tubes	50

## Materials to Be Supplied by the User

1. Microcentrifuge
2. Water bath
3. 1.5 ml or 2 ml centrifuge tubes
4. 100 µl and 1,000 µl pipettors and tips
5. 80% isopropanol or 80% ethanol
6. Ultrapure water or TE buffer

## Protocol

### A. Purification of DNA amplified in PCR reactions

1. Add 300 µl of NE binding buffer to a 50~100 µl PCR reaction or other enzymatic reactions and mix thoroughly. Transfer the mixture to a Miniprep spin column with a 2 ml Collection tube. Let it stand for at least

5 minutes. Centrifuge at 13,000 rpm for 10~20 seconds and discard the flow-through.

2. Add 500  $\mu$ l of 80% isopropanol (or 80% ethanol) to the Spin column. or ethanol.
3. Place the Spin column into a new 1.5 ml microtube. Let the tube lid open for 2~3 minutes to volatilize ethanol completely.
4. Add 40~50  $\mu$ l TE buffer or ultrapure water into the center part of the SiMax™ membrane in the Spin column. Incubate at room temperature for 3~5 minutes. Centrifuge at 13,000 rpm for 1 minute to elute DNA.
5. **Note:** Repeat this step once if more DNA is required. Centrifuge at 13,000 rpm for 30 seconds and discard the flow-through.
6. Repeat the wash procedure in step 2.  
Centrifuge at 13,000 rpm for an additional 1 minute to remove the residual isopropanol.
7. Determine the quality of the purified DNA fragment on 1% agarose gel stained with GoldView™ or EB. Store the DNA fragment at 4°C for immediate use or at -20 °C for future use.

## **B. Extraction of DNA fragment from agarose gel**

1. Excise the DNA fragment from the agarose gel with a clear, sharp scalpel. Weigh the gel slice and transfer it to a 1.5 ml centrifuge tube.
2. Add 300  $\mu$ l of NE binding buffer to the centrifuge tube containing 100 mg gel slice. Incubate at 50~60°C for 3~5 minutes and invert the tube occasionally until the agarose gel is completely dissolved.
3. Transfer the above mixture to a Miniprep spin column with a 2 ml Collection tube. Let it stand for 5 minutes. Centrifuge at 13,000 rpm for 10~20 seconds and discard the flow-through.
4. Add 500  $\mu$ l of 80% isopropanol (or 80% ethanol) to the Spin column. Centrifuge at 13,000 rpm for 30 seconds and discard the flow-through.
5. Repeat the washing procedure in step 4.

6. Centrifuge at 13,000 rpm for an additional 1 minute to remove the residual isopropanol or ethanol.
7. Place the Spin column into a new 1.5ml microtube. Let the tube lid open for 2~3 minutes to volatilize ethanol completely.
8. Add 40~50  $\mu$ l TE buffer or ultrapure water into the center part of the SiMax<sup>™</sup> membrane in a Spin column. 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 DNA fragment on 1% agarose gel stained with GoldView<sup>™</sup> or EB. Store the purified DNA at 4 °C for immediate use or at -20 °C for future use.

# SiMax™ Genomic DNA Extraction

## Features

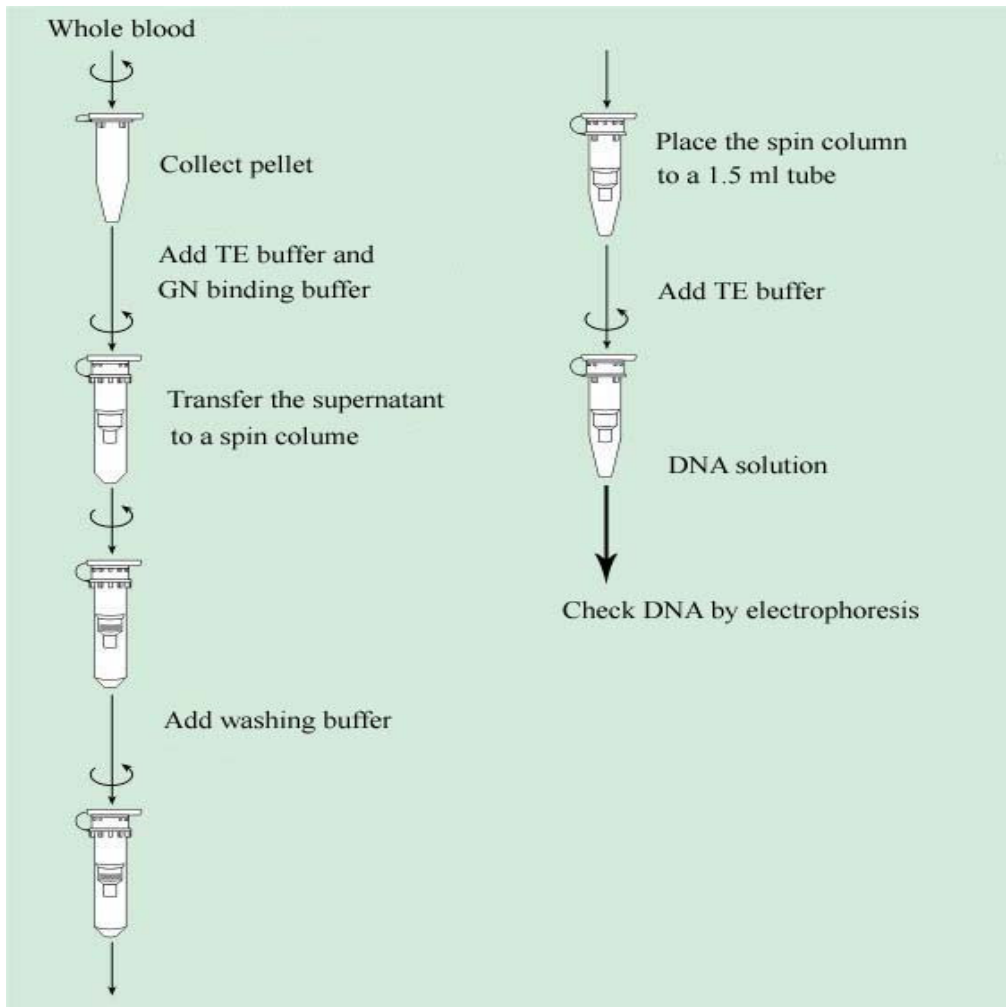
**Rapid:** Rapid extraction of genomic DNA in less than 30 minutes.

**Simple:** No need for proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation.

**High yields:** Purify up to 20~40 µg genomic DNA per ml of whole blood.

**High quality:** Pure DNA, free from contaminants and enzyme inhibitors, is suitable for a variety of molecular biology application.

**Wide application:** For the isolation of genomic DNA from whole blood as well as from many other samples.



# SiMax™ Genomic DNA Extraction User's Instruction

## 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 be also 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

	<u>50 preps</u>
6. GN binding buffer	50ml
7. Washing buffer	50ml
8. Miniprep spin column with 2 ml collection tubes	50

## Notes before use

When the room temperature is below 25°C, 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°C 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  $\mu$ l 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  $\mu$ l 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 GoldView™ or EB. Store the purified DNA at 4 °C for immediate use or at -20°C 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  $5 \times 10^6$  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  $5 \times 10^6$  cells) by centrifugation at 13,000 rpm for 30 seconds. Resuspend the bacterial cells in 400  $\mu$ l of 50 mM EDTA solution. Add 50  $\mu$ l of 100 mg/ml lysozyme to the suspension and incubate at 37°C for 30~60 min, shaking gently. Then add 250  $\mu$ l of STEP solution and shake gently at 50°C for at least 2 hours. 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**

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

- 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  $5 \times 10^6$  cells) by centrifugation at 13,000 rpm for 30 seconds. Resuspend the cells in 500  $\mu$ l 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°C. Incubate at 65°C for 15~20 min. 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 Na<sub>2</sub>HPO<sub>4</sub> and 0.024g KH<sub>2</sub>PO<sub>4</sub> in 80 ml ultrapure water. Adjust the pH to 7.4 with HCl. Add ultrapure water too 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.

#### **STEP Solution: 100 ml**

Mix 0.25 ml of 20% SDS, 15 ml of 1 mol/L Tris-HCl (pH8.0), 80ml of 0.5 mol/L EDTA (pH8.0), and 1.25 ml of 20 mg/ml Proteinase K together. Add ultrapure water to 100 ml. The solution can be sterilized by filter and stored at -20°C.