5. Add 500 μL Buffer GW2 to the adsorption column (check whether ethanol has been added before use), centrifuge at 12,000 rpm for 1 minute, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.
   **Note: To further improve DNA purity, repeat step 5.**
6. Centrifuge at 12,000 rpm for 2 minutes and discard the waste liquid in the collection tube. Allow the cartridge to dry at room temperature for several minutes.
   **Note: The purpose of this step is to remove the residual ethanol in the adsorption column, and the residual ethanol will affect the subsequent enzymatic reactions (enzyme digestion, PCR, etc.).**
7. Put the adsorption column in a new centrifuge tube (self-provided), add 50-200 μL Buffer GE or sterilized water to the middle of the adsorption column, leave it at room temperature for 2-5 minutes, centrifuge at 12,000 rpm for 1 minute,Collcet DNA solution and store at -20°C.
   **Note: 1) If the downstream experiment is sensitive to pH or EDTA, it can be eluted with sterile water. The pH value of the eluent has a great influence on the elution efficiency. If water is used as the eluent, the pH value should be ensured to be between 7.0 and 8.5 (the pH value of the water can be adjusted to this range with NaOH), and the pH lower than 7.0 will result in low elution efficiencye.**
   **2) Preheat Buffer GE in a water bath at 65-70°C, and incubate at room temperature for 5 minutes before centrifugation to increase yield; elute with another 50-200 μL of Buffer GE or sterile water to increase yield.**
   **3) If you want to increase the final concentration of DNA, you can re-add the obtained solution to the adsorption column, place at room temperature for 2-5 minutes, and centrifuge at 12,000 rpm for 1 minute; if the elution volume is less than 200 μL, you can increase the final concentration of DNA, but may reduce total production. If the amount of DNA is less than 1 μg, it is recommended to elute with 50 μL Buffer GE or sterile water.**
   **4) Due to DNA stored in water will be affected by acid hydrolysis, if it needs to be stored for a long time, it is recommended to use Buffer GE to elute and store at -20°C.**




**Version: 12/2023**

# Universal Genomic DNA Kit

**Cat. No. :** CW2298S (50 preps)
CW2298M (200 preps)

**Storage Conditions:** Store at room temperature (15-30°C).

## Components

| Component | CW2298S 50 preps | CW2298M 200 preps |
|---|---|---|
| Buffer GTL | 15 mL | 60 mL |
| Buffer GL | 15 mL | 50 mL |
| Buffer GW1（concentrate） | 13 mL | 52 mL |
| Buffer GW2（concentrate） | 15 mL | 70 mL |
| Buffer GE | 15 mL | 60 mL |
| Proteinase K | 1.25 mL | 4×1.25 mL |
| Spin Columns DM with Collection Tubes | 50 | 200 |

## Introduction

This kit is suitable for extracting high-purity total DNA from fresh or frozen animal tissues, cells, blood, bacteria and other samples. The size of purified DNA fragment is up to 50 kb by this product. The purification process does not require toxic solvents such as phenol or chloroform, and ethanol precipitation. The kit uses an optimized buffer system to efficiently and specifically bind DNA to the adsorption column and remove inhibitors from PCR and enzymatic reactions by two-step washing.Then High-purity DNA can be obtained by elution with low-salt buffer or water. The purified DNA can be directly used in downstream experiments such as enzyme digestion, PCR, Real-Time PCR, library construction, Southern Blot, and molecular markers.

## Reagents to Be Supplied by User

100% ethanol

Enzymatic Lysis Buffer (must be prepared for the extraction of gram-positive bacterial genomic DNA).

Enzymatic Lysis Buffe formulation: 20 mM Tris, pH 8.0; 2 mM $Na_2$-EDTA; 1.2% TritonX-100; final concentration of Lysozyme is 20 mg/mL.

## Precautions

1. Repeated freezing and thawing of samples should be avoided, otherwise the extracted DNA fragments will be smaller and the lower extraction yield.
2. If extracting genomes from bacterial cultures with substantial accumulation of secondary metabolites or with thick cell walls, it is recommended to collect samples early in the logarithmic growth phase.
3. Add absolute ethanol to Buffer GW1 and Buffer GW2 according to the instructions on the reagent bottle label before the first use.



(1) Take 1-5 mL of bacterial culture ($10^6$-$10^8$ cells, no more than $2\times10^9$ cells) into a centrifuge tube (self-provided), and centrifuge at 12,000 rpm (~13,400 × g) for 1 minute, as far as possible Aspirate the supernatant.

(2) Add 180 μL Buffer GTL to the pellet and shake to resuspend the bacteria.

(3) Add 20 μL Proteinase K, mix by vortexing, and incubate at 56°C until the cells are completely lysed. During the incubation, invert or shake the centrifuge tube at intervals to disperse the samples.

**Note: To remove RNA, after the above steps are completed, add 4 μL of 100 mg/mL RNase A solution (Cat. No.: CW0601S), shake and mix, and leave at room temperature for 5-10 minutes.**

(4) Add 200 μL Buffer GL, and mix by vortexing.

1b. Gram-positive bacteria

(1) Take 1-5 mL of bacterial culture ($10^6$-$10^8$ cells, no more than $2\times10^9$ cells) into a centrifuge tube (self-provided), centrifuge at 12,000 rpm for 1 minute, and aspirate the supernatant as much as possible.

(2) Add 180 μL Enzymatic Lysis Buffer (self-prepared) to resuspend the bacteria.

(3) Incubate at 37°C for 30 minutes.

(4) Add 20 μL Proteinase K, vortex and mix well. Add 200 μL Buffer GL, and mix by vortexing. Incubate at 56°C for 30 minutes.

**Note: 1) If desired, 15 min incubation at 95°C can inactivate pathogens, but 95°C incubation will cause some DNA degradation.**

**2) To remove RNA, after the above steps are completed, add 4 μL of 100 mg/mL RNase A solution (Cat. No.: CW0601S), shake and mix, and leave at room temperature for 5-10 minutes.**

2. Add 200 μL ethanol and vortex to mix well.

   **Note: A white precipitate may occur after adding absolute ethanol, which will not affect subsequent experiments.**
3. Add the solution obtained in step 2 (including the formed precipitate) to the adsorption column (Spin Columns DM) that has been loaded into the collection tube. If the solution cannot be added at one time, it can be transferred in multiple times. Centrifuge at 12,000 rpm for 1 minute, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.
4. Add 500 μL Buffer GW1 to the adsorption column (check whether ethanol has been added before use), centrifuge at 12,000 rpm for 1 minute, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

5. Add 500 μL Buffer GW1 to the adsorption column (check whether ethanol has been added before use), centrifuge at 12,000 rpm for 1 minute, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.
6. Add 500 μL Buffer GW2 to the adsorption column (check whether absolute ethanol has been added before use), centrifuge at 12,000 rpm for 1 minute, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

   **Note: To further improve DNA purity, repeat step 6.**
7. Centrifuge at 12,000 rpm for 2 minutes and discard the waste liquid in the collection tube. Allow the cartridge to dry at room temperature for several minutes.

   **Note: The purpose of this step is to remove the residual ethanol in the adsorption column, and the residual ethanol will affect the subsequent enzymatic reactions (enzyme digestion, PCR, etc.).**
8. Put the adsorption column in a new centrifuge tube (self-provided), add 50-200 μL Buffer GE or sterilized water to the middle of the adsorption column, leave it at room temperature for 2-5 minutes, centrifuge at 12,000 rpm for 1 minute, Collect the DNA solution and store at -20°C.

   **Note: 1) If the downstream experiment is sensitive to pH or EDTA, it can be eluted with sterile water. The pH value of the eluent has a great influence on the elution efficiency. If water is used as the eluent, the pH value should be ensured to be between 7.0 and 8.5 (the pH value of the water can be adjusted to this range with NaOH), and the pH lower than 7.0 will result in low elution efficiency.**

   **2) Preheat Buffer GE in a water bath at 65-70°C, and incubate at room temperature for 5 minutes before centrifugation to increase the yield; elute with another 50-200 μL of Buffer GE or sterile water to increase the yield.**

   **3) If you want to increase the final concentration of DNA, you can re-add the obtained solution to the adsorption column, place at room temperature for 2-5 minutes, and centrifuge at 12,000 rpm for 1 minute; if the elution volume is less than 200 μL, you can increase the final concentration of DNA, But may reduce total production. If the amount of DNA is less than 1 μg, it is recommended to elute with 50 μL Buffer GE or sterile water.**

   **4) Because DNA stored in water will be affected by acid hydrolysis, if it needs to be stored for a long time, it is recommended to use Buffer GE to elute and store at -20°C.**

### iii Bacterial Genome Extraction

1. Bacterial sample pretreatment

   1a. Gram-negative bacteria



4. Before use, please check whether Buffer GTL and Buffer GL are crystallized or precipitated. If there is crystallisation or precipitation, please re-dissolve Buffer GL and Buffer GTL in a 56°C water bath.
6. If the downstream experiment is sensitive to RNA contamination, add 4 μL DNase-Free RNase A (100 mg/mL) before adding Buffer GL. RNase A is not provided in this kit. If necessary, you can order it from our company separately. Catalog : CW0601S.

## Protocol

### i Genome extraction from blood and cell samples

1. material handling

   1a. If the extraction material is mammalian anticoagulated blood (enucleated red blood cells), directly add Buffer GTL to 50-200 μL fresh or frozen anticoagulated blood sample to make up to 200 μL;

   1b. If the extraction material is anticoagulated blood from birds, birds, amphibians or lower organisms, and the red blood cells are nucleated cells, take 5-10 μL of fresh or frozen anticoagulated blood samples, add Buffer GTL to make up to 200 μL;

   1c. The adherent cultured cells should be processed into a cell suspension (the maximum extraction volume is $5\times10^6$ cells), centrifuged at 2,000 rpm (400×g) for 5 minutes, discarded the supernatant, added 200 μL GTL, and shaken to the sample completely suspended;

   **Note: To remove RNA, after the above steps, add 4 μL of 100 mg/ml RNase A solution (Cat. No.: CW0601S), vortex for 15 seconds, and leave at room temperature for 2 minutes.**
2. Add 20 μL Poteinase K.
3. Add 200 μL Buffer GL, vortex to mix well, and Incubate at 56 °C for 10 min.
4. Briefly centrifuge to remove beads of water from the inside of the tube lid. Add 200 μL ethanol and vortex to mix well. Centrifuge briefly.

   **Note: 1) Vortex immediately after adding Buffer GL and ethanol.**

   **2) After adding Buffer GL and absolute ethanol, a white precipitate may occur, which will not affect subsequent experiments. Some tissues may form a sol-like product after adding Buffer GL and absolute ethanol, and vigorous shaking or vortexing is recommended.**
5. All the solution obtained in the previous step is added to the (Spin Columns DM) which has been loaded into the collection tube. If the solution cannot be added at one time, it can be transferred in multiple times. Centrifuge at 12,000 rpm (~13,400 × g) for 1 minute, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

6. Add 500 μL Buffer GW1 to the adsorption column (check whether ethanol has been added before use), centrifuge at 12,000 rpm for 1 minute, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.
7. Add 500 μL Buffer GW2 to the adsorption column (check whether ethanol has been added before use), centrifuge at 12,000 rpm for 1 minute, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

   **Note: To further improve DNA purity, repeat step 7.**
8. Centrifuge at 12,000 rpm for 2 minutes and discard the waste liquid in the collection tube. Allow the cartridge to dry at room temperature for several minutes.

   **Note: The purpose of this step is to remove the residual ethanol in the adsorption column, and the residual ethanol will affect the subsequent enzymatic reactions (enzyme digestion, PCR, etc.).**
9. Put the adsorption column in a new centrifuge tube (self-provided), add 50-200 μL Buffer GE or sterilized water to the middle of the adsorption column, leave it at room temperature for 2-5 minutes, centrifuge at 12,000 rpm for 1 minute, and collect the DNA solution, and store at -20°C.

   **Note: 1) If the downstream experiment is sensitive to pH or EDTA, it can be eluted with sterile water. The pH value of the eluent has a great influence on the elution efficiency. If water is used as the eluent, the pH value should be ensured to be between 7.0 and 8.5 (the pH value of the water can be adjusted to this range with NaOH), and the pH lower than 7.0 will result in low elution efficiency.**

   **2) Preheat Buffer GE in a water bath at 65-70°C, and incubate at room temperature for 5 minutes before centrifugation to increase the yield; elute with another 50-200 μL of Buffer GE or sterile water to increase the yield.**

   **3) If you want to increase the final concentration of DNA, you can re-add the obtained solution to the adsorption column, place at room temperature for 2-5 minutes, and centrifuge at 12,000 rpm for 1 minute; if the elution volume is less than 200 μL, you can increase the final concentration of DNA, but may reduce total yield. If the amount of DNA is less than 1 μg, it is recommended to elute with 50 μL Buffer GE or sterile water.**

   **4) Due to DNA stored in water will be affected by acid hydrolysis, if long-term storage, it is recommended to use Buffer GE to elute and store at -20°C.**

### ii Animal tissue genome extraction

1. material handling

   If the extraction material is animal tissue, take 25 mg (the amount of spleen tissue should be less than 10 mg); if the material is rat tail, take a piece of rat tail with a length of 0.4-0.6 cm or two pieces with a length of 0.4-0.6 cm mouse tail.



   1a. The samples are ground in liquid nitrogen or cut into small pieces and placed in a 1.5 mL centrifuge tube, and add 180 μL Buffer GTL to tube, mark different samples well.

   1b. If using a homogenizer to process the sample, add no more than 80 μL Buffer GTL to the sample before homogenizing, and add 100 μL Buffer GTL after homogenizing.

   **Note: 1) Make sure that the amount of each tissue does not exceed the recommended range.**

   **2) Tissue samples are ground with liquid nitrogen before addition of Buffer GTL or homogenized with a homogenizer after addition of Buffer GTL, which can increase the lysis efficiency.**
2. Add 20 μL Proteinase K, mix thoroughly by vortexing. Incubate at 56 °C until the tissue is completely lysed. During incubation, the sample can be dispersed by inverting or shaking the centrifuge tube at intervals.

   **Note: 1) Different tissues have different digestion time, usually 1-3 hours to complete, mouse tail needs to be digested for 6-8 hours, if necessary, overnight digestion will not affect subsequent operations.**

   **2) If there is still a gelatinous substance after incubation and vortexing, extend the incubation time of 56°C or add 20 μL of Proteinase K to digest.**

   **3) To remove RNA, after the above steps are completed, add 4 μL of 100 mg/ml RNase A solution (Cat. No.: CW0601S), vortex for 15 seconds, and leave at room temperature for 5-10 minutes.**
3. Add 200 μL Buffer GL, mix well by vortexing, and Incubate at 70°C for 10 minutes. Centrifuge briefly, add 200 μL ethanol, mix thoroughly by vortexing.

   **Note: 1) Vortex immediately after addition of Buffer GL and absolute ethanol.**

   **2) After addition of Buffer GL and absolute ethanol, a white precipitate may occur, which will not affect subsequent experiments. Some tissues (eg, spleen, lung) may form a sol-like product after addition of Buffer GL and absolute ethanol. In this case, vigorous shaking or vortexing is recommended.**
4. Centrifuge briefly, and add all the solution obtained in step 3 to the adsorption column (Spin Columns DM) that has been loaded into the collection tube. If the solution cannot be added at one time, it can be transferred in multiple times. Centrifuge at 12,000 rpm (~13,400 × g) for 1 minute,Discard the waste liquid in the collection tube and put the adsorption column back into the collection tube.