7. Add 500 μL of Buffer GW1 to the adsorption column (check whether 100% ethanol is 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: Step 7 is recommended to be repeated if the extracted sample is the blood genome of a species such as mouse or monkey where heme removal is difficult.**

8. Add 500 μL of Buffer GW2 to the adsorption column (check whether 100% ethanol is 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 8.**

9. Centrifuge at 12,000 rpm for 2 minutes and discard the waste 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. The residual ethanol will affect the subsequent enzymatic reactions (enzyme digestion, PCR, etc.).**

10. Put the adsorption column in a new centrifuge tube (self-provided), add 50-200 μL Buffer GE or RNase-Free 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 , DNA was stored at -20°C.

    **Note: 1) If the downstream experiment is sensitive to pH or EDTA, it can be eluted with** RNase-Free **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 value should be lower than 7.0 The elution efficiency is not high.**

    **2) If you want to increase the final concentration of DNA, you can re-add the obtained DNA eluate to the adsorption membrane, place at room temperature for 2-5 minutes, and centrifuge at 12,000 rpm for 1 minute.**

    **3) 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.**




**Version: 12/2023**

# Blood Genomic DNA Mini Kit (0.1-1 mL)

**Cat. No. :** CW2087S (50 preps)
CW2087M (200 preps)

**Storage Conditions:** Store Buffer RCL at 2-8℃. All rest components should be stored at room temperature (15-30℃). It can be transported at room temperature, and the transport time is recommended not to exceed 7 days.

## Components

| Component | CW2087S 50 preps | CW2087M 200 preps |
|---|---|---|
| Buffer RCL | 125 mL | 2×260 mL |
| Buffer GR | 15 mL | 50 mL |
| Buffer GL | 15 mL | 50 mL |
| Buffer GW1（concentrate） | 13 mL | 52 mL |
| Buffer GW2（concentrate） | 13 mL | 50 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 samples from fresh or frozen whole blood (blood samples treated with anticoagulants such as citrate, EDTA, or heparin), plasma, serum, buffy coat, lymphocytes, cell-free body fluids, etc. Total DNA was extracted, including genomic DNA, mitochondrial DNA and viral DNA. This product can process 0.1-1 mL of whole blood, and the highest yield can reach 30 μg. It can purify DNA with a size of 100 bp to 50 kb. The purified DNA has high yield and good quality, and can remove proteins, pigments and lipids to the maximum extent. It can be directly used in PCR, real-time quantitative PCR, enzyme digestion and Southern Blot experiments.

## Reagents to Be Supplied by User

100% ethanol

## Precautions

1. Repeated freezing and thawing of samples should be avoided, otherwise the extracted DNA fragments will be smaller and the extraction yield will also decrease.
2. This kit can extract up to 0.1-1 mL whole blood sample or $1\times10^7$ leukocytes.
3. Before the first use, add 100% ethanol to Buffer GW1 and Buffer GW2 according to the instructions on the reagent bottle label.
4. Before use, please check whether the Buffer GL has crystals or precipitates. If there are crystals or precipitates, please re-dissolve the Buffer GL by incubating in a 56°C water bath.
5. The Buffer RCL in the kit cannot be used after it is cloudy.



## Protocol

1. Sample processing

   **1a.** When extracting a 200 uL blood sample, add the sample to the centrifuge tube (self-prepared) and proceed directly to the next experiment.

   **1b.** When the blood sample volume is less than 200 μL, add Buffer GR to make up to 200 μL, and then proceed to the next experiment.

   **1c.** When the blood sample volume exceeds 200 μL, add 1-2.5 times the volume of Buffer RCL, gently vortex or invert to mix, centrifuge at 12,000 rpm (~13,400 × g) for 1 minute, and carefully remove the supernatant. There is still red in the precipitate, you can repeat the above steps once. Then add 200 μL of Buffer GR to the precipitate, shake until thoroughly mixed, and then proceed to the next experiment.

   **1d.** If the processed blood sample is anticoagulant from poultry, birds, amphibians or lower organisms, its red blood cells are nucleated cells, and the blood sample volume is 5-20 μL, Buffer GR can be added to make up to 200 μL before proceeding. follow-up experiments.

   **Note: If the downstream assay is sensitive to RNA, add 4 μL of RNase A (100 mg/mL) solution, shake for 15 seconds, and leave at room temperature for 5 minutes. RNase A is not provided in this kit. If necessary, it can be ordered from our company separately, item number: CW0601S.**
2. Add 20 μL Proteinase K to the above solution and mix well.
3. Add 200 μL Buffer GL and shake until thoroughly mixed.

   **Note: Do not premix Proteinase K and Buffer GL.**
4. Incubate at 56°C for 10 minutes, invert and mix several times.

   **Note: DNA yields have been maximized after 10 minutes of incubation, and further incubation times have no effect on DNA yield and purity.**
5. Add 200 μL of 100% ethanol and mix by inversion several times. Briefly centrifuge to concentrate the liquid on the tube walls and wall caps to the bottom of the tube.
6. Add all the solution obtained in step 5 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.