Website: www.cwbiosciences.com
Technical Support: service@cwbiosciences.com
Customer Service: 617-800-6495

Blood Genomic DNA Mini Kit (0.1-1 ml)

Catalog Number: CW2087S (50 preps)

CW2087M (200 preps)

Storage Condition: store Buffer RCL at 2-8°C, other components at room temperature (15-30°C).

Kit Components:

Component	CW2087S (50 preps)	CW2087M (200 preps)
Buffer RCL	125 ml	2x260 ml
Buffer GR	15 ml	50 ml
Buffer GL	15 ml	50 ml
Buffer GW1 (concentrated)	13 ml	52 ml
Buffer GW2 (concentrated)	15 ml	50 ml
Buffer GE	15 ml	60 ml
Proteinase K	12.5 mg	50 mg
Proteinase K Storage Buffer	1.25 ml	5 ml
Spin Columns DM with Collection Tubes	50	200

Product Introduction:

The kit is suitable for extracting total DNA including genomic DNA, mitochondria DNA and virus DNA, from fresh or frozen anticoagulated blood (blood samples treated with citrate, EDTA, heparin), plasma, serum, buffy coat, lymphocytes, and cell-free body fluids, etc.

This product can handle 0.1-1 ml of whole blood with a maximum yield of 30 μg and can be used to purify DNA ranging from 100 bp to 50 kb. The purified DNA has high yield and excellent quality, which has been maximumly removed of contaminations such as protein, pigment, lipid and other inhibitory impurities. The purified DNA can be directly used in experiments such as PCR, real-time PCR, enzyme digestion and Southern Blot.

Not included in the kit: 100% ethanol.

Preparation before the experiment and important notes:

1. Add designated amount of Proteinase K Storage Buffer to the proteinase K powder to dissolve it, then store it at -20°C. The prepared Proteinase K solution should not be left at room temperature for a long time and avoid repeated freezing and thawing so as not to affect its activity.

Cat. No.	CW2087S	CW2087M
Proteinase K Storage Buffer	1.25 ml	5 ml

2. The samples should avoid repeated freezing and thawing, which will result in smaller DNA fragments and lower yield.
3. The kit can handle up to 0.1-1 ml of whole blood sample or 1 x 10^7 white blood cells.
4. Before the first experiment, add the specified amount of 100% ethanol to Buffer GW1 and Buffer GW2 according to the instructions on the reagent bottle label and mark it.
5. Check if precipitation occurs in the Buffer GL before use. If yes, re-dissolve in a 56°C water bath.
6. The Buffer RCL in the kit cannot be used after it has become cloudy.

Protocol:

1. Sample treatment:

1a. For 200 ul of blood sample, add the sample to the centrifuge tube (self-prepared) and proceed directly to the next step.

1b. If the blood sample is less than 200 ul, add Buffer GR to 200 ul, then proceed to the next step.

1c. If the blood sample is more than 200 ul, add 1-2.5 x volume of Buffer RCL. Vortex gently or invert the tube upside down to mix

well. Centrifuge at 12,000 rpm (~13,400 xg) for 1 minute and discard the supernatant carefully. If the precipitation is still red, repeat above step once. Then add 200 ul Buffer GR to the precipitation. Vortex to mix thoroughly, then proceed to the next step.

1d. If the sample is anticoagulated blood from poultry, birds, amphibians or lower organisms, the red blood cells are nucleated cells. Take 5-10 µl of blood sample and add Buffer GR to make it to 200 µl, then proceed to the next step.

Note: If downstream experiments are sensitive to RNA, add 4 ul 100 mg/ml RNase A (Cat. No. CW0601S) solution after above steps; Vortex for 15 seconds and leave at room temperature for 5 minutes.

2. Add 20 ul Proteinase K to above solution and vortex to mix thoroughly.
3. Add 200 ul Buffer GL, then vortex to mix thoroughly.
   Note: don't pre-mix Proteinase K and Buffer GL.
4. Incubate at 56°C water bath for 10 minutes, inverting the tube upside down serval times internally to mix well.
   Note: Maximum yield of DNA can be achieved by incubation for 10 minutes; extension of incubation time has no effect on the yield and purity of DNA.

5. Add 200 ul 100% ethanol and invert the tube upside down several times. Centrifuge briefly to collect all the liquid to the bottom of the tube.
6. Transfer the solution obtained from step 5 to a column with collection tube (Spin Columns DM); Transfer multiple times if needed; Centrifuge at 12,000 rpm for 1 minute; Discard the waste in the collection tube and put the column back to the collection tube.
7. Add 500 µl Buffer GW1 to the column (check if 100% ethanol has been added before use). Centrifuge for 1 minute at 12,000 rpm. Discard the waste from the collection tube and put back the column to the collection tube.

   Note: If the sample is the blood of a species whose hemoglobin is difficult to remove, such as a mouse or a monkey, it is recommended to repeat step 7.
8. Add 500 µl Buffer GW2 to the column (check if 100% ethanol has been added before use). Centrifuge for 1 minute at 12,000 rpm. Discard the waste from the collection tube and put back the column to 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 from the collection tube. Leave the column at room temperature to dry thoroughly.

Note: The purpose of this step is to remove the residual ethanol in the column, and the residual of ethanol will affect the downstream enzymatic reactions (enzyme digestion, PCR, etc.).

10. Place the column in a new 1.5 ml centrifuge tube (self-prepared) and add 50-200 μl of Buffer GE or autoclaved H_2O in the middle of the membrane and allow it to stand at room temperature for 2-5 minutes. Centrifuge at 12,000 rpm for 1 minute and collect the DNA solution. Store the DNA at -20°C.

    Note:

    1) If downstream experiments are sensitive to pH or EDTA, autoclaved H_2O can be used for elution. The pH of the elution buffer has a profound influence on the elution efficiency. If H_2O is used, the pH should be 7.0-8.5 (NaOH can be used to adjust the pH of H_2O to this range). If the pH is lower than 7.0, the elution efficiency will be low.
    2) To increase the final DNA concentration, the DNA elution from step 10 can be added back to the column and leave at room temperature for 2-5 minutes, then centrifuge at 12,000 rpm for 1 minute.
    3) Because the DNA stored in water is affected by acidic hydrolysis, for long-term storage, it is recommended to elute with Buffer GE and store at -20°C.