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

Universal Genomic DNA Kit

Catalog Number: CW2298S (50 preps)
CW2298M (200 preps)

Storage Condition: room temperature (15-30°C).

Kit Components:

Component	CW2298S (50 preps)	CW2298M (200 preps)
Buffer GTL	15 ml	60 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	25 mg	90 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 of high-purity from fresh or frozen animal tissues, cells, blood, bacteria and other samples. This product can be used to obtain a DNA fragment up to 50 kb. The purification process does not require the use of toxic solvents such as phenol or chloroform and does not require ethanol precipitation.

The optimized buffer system allows the DNA after lysis to be efficiently and specifically bound to the silica matrix column. The inhibitors of PCR and other enzymatic reactions can be effectively removed by two-step washings, and finally high-purity DNA is obtained by elution with a 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.

Not Included in The Kit:

1. 100% ethanol;
2. Enzymatic Lysis Buffer (Prepare for extraction of gram-positive genomic DNA);
   Enzymatic Lysis Buffer receipt: 20 mM Tris (pH8.0); 2 mM Na_2-EDTA; 1.2% Triton X-100; Lysozyme (final concentration: 20 mg/ml).

Preparation before the experiment and important notes:

1. Add specific 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.

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Proteinase K Storage Buffer	1.25 ml	4.5 ml

2. The sample should avoid repeated freezing and thawing, otherwise it will result in smaller extracted DNA fragments and low yield.
3. If extracting the genome of a bacterial culture in which a lot of secondary metabolites accumulate or bacteria with thick cell wall, it is recommended to collect the samples at early of the logarithmic growth phase.
4. Before the first use, 100% ethanol should be added to Buffer GW1 and Buffer GW2 according to the instructions on the label of the reagent bottle. Mix well and mark the label.
5. Before use, check if there are crystals or precipitation in Buffer GTL and Buffer GL. If yes, put Buffer GL and Buffer GTL in a 56°C water bath to re-dissolve.

6. If downstream experiments are sensitive to RNA contamination, 4 μl of DNase-Free RNase A (100 mg/ml) can be added before Buffer GL is added. RNase A is not included in this kit and it can be ordered separately from our company (Cat. No.: CW0601S).

Protocol:

I Extraction of genomic DNA from blood and cell samples"

1. Sample treatment:

   1a. If the sample is mammalian anticoagulated blood (nucleated red blood cells), Buffer GTL can be added directly to 50-200 μl fresh or frozen anticoagulated blood sample to make it to 200 μl;

   1b. 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 fresh or frozen anticoagulated blood sample, and add Buffer GTL to make it to 200 μl;

   1c. Resuspend the attached cells first (maximum $5x10^6$ cells), then centrifuge at 2,000rpm (400xg) for 5 minutes. Discard the supernatant and add 200 ul Buffer GTL. Shake until the sample is resuspended thoroughly.

Note: To remove RNA, add 4 ul 100 mg/ml RNase A solution after above steps; Vortex for 15 seconds, and leave at room temperature for 2 minutes.

2. Add 20 ul Proteinase K.
3. Add 200 ul Buffer GL; Vortex to mix thoroughly, then incubate at 56°C water bath for 10 minutes.
4. Centrifuge briefly; Add 200 ul 100% ethanol, then vortex to mix thoroughly; Centrifuge briefly.

   Note: 1) Vortex immediately after adding Buffer GL and 100% ethanol.

   2) White precipitate may be generated after addition of Buffer GL and 100% ethanol without affecting subsequent results.
5. Transfer the solution obtained from above step to a column with collection tube (Spin Columns DM); Transfer multiple times if needed; Centrifuge at 12,000 rpm (~13,400 xg) for 1 minute; Discard the waste in the collection tube and put the column back to the collection tube.
6. 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.
7. 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 7.

8. Centrifuge at 12,000 rpm for 2 minutes and discard the waste from the collection tube.

   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.).

9. 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) Pre-warm of Buffer GE at 65-70°C water bath, and incubation at room temperature for 5 minutes will increase

the yield. The yield can be also increased by elution with another 50-200 µl of Buffer GE or sterile H_2O.

3) To increase the final DNA concentration, the DNA elution from step 10 can be added back to the column and repeat step 10; if the elution volume is less than 200 µl, the final DNA concentration can be increased, but the total yield may be reduced. If the amount of DNA is less than 1 µg, it is recommended to elute with 50 µl Buffer GE or sterile H_2O.
4) 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.

II. Extraction of genomic DNA from animal's tissues:

1. Sample treatment:

   If the sample is animal's tissue, take 25 mg (for spleen tissue, take less than 10 mg); for mice tails, take a 0.4-0.6 cm rat tail or two 0.4-0.6 cm rat tails.

   1a. Samples are ground in liquid nitrogen or cut into small pieces and placed in a 1.5 ml centrifuge tube. Add 180 ul of Buffer GTL and mark different samples well.

   1b. If using homogenizer to treat sample, before homogenization, add Buffer GTL no more than 80 ul to the sample. After homogenization, add 100 ul Buffer GTL.

Note: 1) make sure the amount of the tissue does not exceed the range recommended.

2) Before adding Buffer GTL, grounding in liquid nitrogen or homogenization will increase the efficiency of lysis.

2. Add 20 ul Proteinase K; Vortex to mix thoroughly, then incubate at 56°C water bath until fully lysated. During incubation, invert the tube upside down or vortex to dispense the sample.

   Note: 1) Different tissues have different digestion time, usually 1-3 hours. It will take 6-8 hours for digestion of rat or mouse tail. Digestion for overnight if necessary, which will not affect the follow-up experiment.

   2) If gel-like material is still present after incubation and vortexing, prolong the incubation time at 56°C or add another 20 µl Proteinase K.

   3) To remove RNA, add 4 ul 100 mg/ml RNase A solution after above steps; Vortex for 15 seconds, and leave at room temperature for 5-10 minutes.

3. Add 200 ul Buffer GL; Vortex to mix thoroughly, then incubate at 70°C water bath for 10 minutes. After brief centrifuge, add 200 ul 100% ethanol, then vortex to mix thoroughly.

   Note: 1) Vortex immediately after adding Buffer GL and 100% ethanol.

2) White precipitate may be generated after addition of Buffer GL and 100% ethanol without affecting subsequent results.

4. After brief centrifuge, transfer the solution obtained from step 3 to a column with collection tube (Spin Columns DM); Transfer multiple times if needed; Centrifuge at 12,000 rpm (~13,400 xg) for 1 minute; Discard the waste in the collection tube and put the column back to the collection tube.
5. 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.
6. 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 6.
7. Centrifuge at 12,000 rpm for 2 minutes and discard the waste from the collection tube.

   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.).

8. 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) Pre-warm of Buffer GE at 65-70°C water bath, and incubation at room temperature for 5 minutes will increase the yield. The yield can be also increased by elution with another 50-200 µl of Buffer GE or sterile H_2O.
   3) To increase the final DNA concentration, the DNA elution from step 10 can be added back to the column and repeat step 10; if the elution volume is less than 200 µl, the final DNA concentration can be increased, but the total yield may be reduced. If the amount of DNA is less than 1 µg, it is recommended to elute with 50 µl Buffer GE or sterile H_2O.

4) 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.

III. Extraction of genomic DNA from bacteria:

1. Pre-treatment of bacteria sample

1a. Gram-negative bacteria:

(1) Take 1-5 ml bacteria culture (10^6-10^8 cells, no more than $2x10^9$) to a centrifuge tube; Centrifuge at 12,000 rpm (~13,400 xg) for 1 minute and discard the supernatant as much as possible.

(2) Add 180 ul of Buffer GTL to the pellet, and vortex to resuspend the pellet.

(3) Add 20 ul Proteinase K; Vortex to mix thoroughly, then incubate at 56°C water bath until fully lysated. During incubation, invert the tube upside down or vortex to dispense the sample.

Note: To remove RNA, add 4 ul 100 mg/ml RNase A solution after above steps; Vortex for 15 seconds, and leave at room temperature for 5-10 minutes.

(4) Add 200 ul Buffer GL and vortex to mix thoroughly.

1b. Gram-negative bacteria:

(1) Take 1-5 ml bacteria culture (10^6-10^8 cells, no more than $2x10^9$) to a centrifuge tube; Centrifuge at 12,000 rpm (~13,400 xg) for 1 minute and discard the supernatant as much as possible.

(2) Add 180 ul of Enzymatic Lysis Buffer (self-prepared) to the pellet, and vortex to resuspend the pellet.

(3) Incubate at 37°C for 30 minutes.

(4) Add 20 ul Proteinase K; Vortex to mix thoroughly. Add 200 ul of Buffer GL and vortex to mix thoroughly. Incubate at 56°C for 30 minutes.

Note: 1) If necessary, incubating at 95°C for 15 minutes can inactivate pathogens, but incubation at 95°C will cause some DNA degradation.

2) To remove RNA, add 4 ul 100 mg/ml RNase A solution after above steps; Vortex for 15 seconds, and leave at room temperature for 5-10 minutes.

2. Add 200 ul 100% ethanol, then vortex to mix thoroughly.
   Note: White precipitate may be generated after addition of 100% ethanol without affecting subsequent results.
3. Transfer the solution obtained from step 2 to a column with collection tube (Spin Columns DM); Transfer multiple times if needed; Centrifuge at 12,000 rpm (~13,400 xg) for 1 minute;

Discard the waste in the collection tube and put the column back to the collection tube.

4. 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.
5. 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 5.
6. Centrifuge at 12,000 rpm for 2 minutes and discard the waste from the collection tube.

   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.).
7. 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) Pre-warm of Buffer GE at 65-70°C water bath, and incubation at room temperature for 5 minutes will increase the yield. The yield can be also increased by elution with another 50-200 µl of Buffer GE or sterile H_2O.
3) To increase the final DNA concentration, the DNA elution from step 10 can be added back to the column and repeat step 10; if the elution volume is less than 200 µl, the final DNA concentration can be increased, but the total yield may be reduced. If the amount of DNA is less than 1 µg, it is recommended to elute with 50 µl Buffer GE or sterile H_2O.
4) 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.