Version: 01/2024

AllPure DNA/RNA/Protein Kit

Cat. No. : CW0591S (50 preps)

Shipping and Storage : Room temperature (15-30°C)

Components

Component	CW0591S (50 preps)
Buffer RL	35 mL
Buffer RW1	40 mL
Buffer RW2 (concentrate)	11 mL
RNase-Free Water	10 mL
Buffer GW1 (concentrate)	13 mL
Buffer GW2 (concentrate)	15 mL
Buffer GE	15 mL
Buffer PZ	60 mL
Buffer PLS	15 mL
Spin Columns DM with Collection Tubes	50
Spin Columns RM with Collection Tubes	50
Collection Tubes	100
RNase-Free Centrifuge Tubes (1.5 mL)	100

Introduction

This kit is suitable for the simultaneous isolation and purification of genomic DNA, total RNA and total protein from the same cell or tissue sample. The purified DNA, RNA and protein can be eluted separately and can be used directly in a variety of downstream molecular biology operations. The kit does not contain toxic substances such as phenol and chloroform, and does not require ethanol precipitation, making it easy and fast to operate. The extracted genomic DNA can be used for PCR, Real-time PCR, Southern Blot, Dot Blot, comparative genomic hybridisation (CGH), gene analysis and SNP analysis; total RNA can be applied to RT-PCR, cDNA synthesis, Nothern Blot, Dot Blot and gene microarray; total protein can be for electrophoresis and Western Blot, etc.

Reagents to be Supplied by user

1. β-mercaptoethanol (newly opened or for RNA extraction)
2. 70% ethanol (prepared with RNase-free water)
3. 100% ethanol

Precautions

1. To prevent RNase contamination, the following aspects should be noted:

1.1 Use RNase-free plastic products and tips to avoid cross-contamination.

1.2 Glassware should be dry-baked at 180°C for 4 hours before use, and plasticware can be soaked in 0.5 M NaOH for 10 min, rinse thoroughly with water and then autoclave.

1.3 Use RNase-free water to prepare the solution.

1.4 The operator should wear a disposable mask and gloves, and change gloves regularly during the experiment.

Protein samples that require SDS-PAGE electrophoresis can be performed as follows

19. Add protein Loading Buffer to the protein sample, denature at 95℃ for 5-10 min, and cool the sample to room temperature.
20. Centrifuge at 12,000 rpm for 1 min and aspirate the supernatant for downstream tests such as SDS-PAGE or Western blot.

Note: 1) The volume of Buffer GE should not be less than 100 μL; Small volume affects recovery efficiency.

2) To increase the yield of DNA, add 100 μL new Buffer GE to the adsorption column and repeat step 13; To increase the concentration of DNA, re-add the DNA eluate obtained in step 13 to the adsorption column and repeat step 13.

Protein extraction

14. Add 1× volume of Buffer PZ to the extracted RNA solution (i.e. solution obtained in step 4), mix thoroughly and leave at room temperature for 10-30 min.
15. Centrifuge at 12,000 rpm for 10 min, discard the supernatant.
16. Add 500 μL 70% ethanol and centrifuge at 12,000 rpm for 1 min, discard supernatant as much as possible.
17. Place the centrifuge tube at room temperature for several minutes to dry the precipitate.

    Note:

    The purpose of this step is to remove residual ethanol, as excessive drying will make protein precipitation difficult to dissolve, and incomplete drying of residual ethanol will affect protein loading.
18. Add 100 μL Buffer PLS to obtain protein solution.

    Note:

    1) Protein samples dissolved by Buffer PLS are suitable for SDS-PAGE and Western Blot, but not for protein quantification by Bradford method, 5% SDS can be used for protein solubilization, or select the appropriate protein lysis buffer according to the downstream test.

    2) The amount of protein lysis buffer to be added is determined by the initial sample volume and the specific requirements of the downstream test.

    3) Lysed proteins can be stored at -20°C for several months or 2-8°C for several days.

2. Samples should avoid repeated freezing and thawing. Samples can be stored in Buffer RL at -70°C for one month.
3. Please add β-mercaptoethanol to Buffer RL before use, 1 mL Buffer RL with 10 μL β-mercaptoethanol. Buffer RL with β-mercaptoethanol can be stored for 1 month at room temperature.
4. 100% ethanol should be added to Buffer RW2, Buffer GW1, and Buffer GW2 according to the instructions on the reagent bottle labels prior to first use.
5. Check Buffer RL for crystals or precipitation before use. If crystals or precipitation are present, re-dissolve in a water bath at 56°C.
6. All centrifugation steps are performed using a benchtop centrifuge at room temperature.

Protocol

1. Material processing

1.1 Cells in wall-mounted culture should first be processed into cell suspension (maximum extraction volume of 10^7). The cell precipitate is collected by centrifugation. Add 600 μL Buffer RL (check that β-mercaptoethanol has been added before use) and the cells are lysed by repeated blowing.

Note: Be sure to discard the culture fluid, otherwise it will affect the lysis and subsequent nucleic acid purification steps.

1.2 Take no more than 30 mg of animal tissue, grind it into fine powder with liquid nitrogen, add 600 μL Buffer RL (check that β-mercaptoethanol has been added before use), or add 600 μL Buffer RL (check that β-mercaptoethanol has been added before use) directly and homogenise.

Note: The homogenization should be sufficient, otherwise the RNA yield will be affected.

2. Centrifuge the solution obtained in the previous step at 12,000 rpm (~13,400 ×g) for 3-5 min, carefully add the supernatant to Spin Columns DM and centrifuge at 12,000 rpm for 30-60 s. Collect the filtrate. Place the Spin Columns DM in a new 2 mL collection tube and leave at room temperature or 4°C for DNA extraction.

   Note: Ensure that no liquid remains on the adsorbent column and repeat centrifugation if necessary until all liquid has passed through the membrane of the adsorbent column.

Total RNA extraction

3. Add 1× volume of 70% ethanol (prepared with RNase-free water) to the filtrate obtained in step 2 and mix well.
4. Add all the solution obtained in the previous step to Spin Columns RM. If you cannot add all the solution at once, you can transfer it in stages. Centrifuge at 12,000 rpm for 20 s and retain the liquid in the collection tube for protein extraction.
5. Place the RM into a new 2 mL collection tube, add 700 μL of Buffer RW1 to the Spin Columns RM, and centrifuge at 12,000 rpm for 20 s, discard the waste solution from the collection tube and put the Spin Columns RM back into the collection tube.
6. Add 500 μL Buffer RW2 (check that 100% ethanol has been added before use) to the Spin Columns RM. Centrifuge at 12,000 rpm for 20 s, discard the waste solution from the collection tube and put the Spin Columns RM back into the collection tube.
7. Repeat step 6.
8. Centrifuge at 12,000 rpm for 2 min and discard the waste solution from the collection tube. Leave the Spin Columns RM at room temperature for a few minutes to dry thoroughly.

   Note: The purpose of this step is to remove residual ethanol in the adsorption column. The residual ethanol can affect subsequent enzymatic reactions (digestion, PCR).

9. Place the Spin Columns RM in a new RNase-free 1.5 mL centrifuge tube, add 30-50 μL RNase-Free Water to the middle part of the Spin Columns RM, leave it at room temperature for 2-5 min, centrifuge at 12,000 rpm for 1 min, collect RNA solution. Store RNA at -70°C to prevent degradation.

   Note: 1) The volume of RNase-Free Wate should not be less than 30 μL. Small volume affects recovery efficiency.

   2) To increase the RNA yield, repeat step 9 with 30-50 μL of new RNase-Free Water.

   3) To increase the RNA concentration, re-add the obtained solution to the adsorption column and repeat step 9.

Genomic DNA extraction

10. Add 500 μL Buffer GW1 (check whether 100% ethanol has been added before use) to the Spin Columns DM. Centrifuge at 12,000 rpm for 20 s, discard the waste solution from the collection tube and put the Spin Columns DM back into the collection tube.
11. Add 500 μL Buffer GW2 (check whether 100% ethanol has been added before use) to the Spin Columns DM, Centrifuge at 12,000 rpm for 2 min, discard the waste solution from the collection tube, and place the Spin Columns DM back into the collection tube.

    Note: To further improve DNA purity, repeat step 11.

12. Centrifuge at 12,000 rpm for 2 min and discard the waste solution from the collection tube. Leave the Spin Columns DM at room temperature for a few minutes to dry thoroughly.

    Note: The purpose of this step is to remove residual ethanol from the Spin Columns DM, which can affect subsequent enzymatic reactions (Enzymatic digestion, PCR, etc.)

13. Place the Spin Columns DM in a new centrifuge tube and add 100 μL Buffer GE to the middle part of the Spin Columns DM. Leave at room temperature for 2-5 min, centrifuge at 12,000 rpm for 2 min, collect the DNA solution and store the DNA at -20°C.