AllPure DNA/RNA/Protein Kit

DNA/RNA/Protein Extraction Kit

Item No. A665492 (50 preps)
Storage conditions: room temperature (15-30° C)

Product content

individual parts making up a compound	A665492 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

Product 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. It is not necessary to divide the sample into three parts to extract DNA, RNA and protein separately, nor to divide the purified total nucleic acid into two parts before purifying DNA and RNA separately. Therefore, it can maximize the recovery of DNA, RNA and protein, and can be used for the purification of nucleic acid and protein in small quantities and rare samples. The purified DNA, RNA and proteins can be eluted separately and can be directly applied to 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 in PCR, Real-time PCR, SouthernBlot, Dot Blot, Comparative Genomic Hybridization (CGH), gene analysis and SNP analysis; total RNA can be applied to RT-PCR, cDNA synthesis, Nothern Blot, Dot Blot and gene microarray experiments; total protein can be be applied to electrophoresis and WesternBlot, etc.

Self-contained reagents: β-mercaptoethanol (freshly opened or for RNA extraction), 70% ethanol (prepared with RNase-free water), Anhydrous ethanol.

Lab prep and important notes:

1. To prevent RNase contamination, attention should be paid to the following aspects:
1) Use RNase-free plastics and tips to avoid cross-contamination.
2) Glassware should be dry baked at 180℃ for 4 hours before use, plasticware can be soaked in 0.5 M NaOH for 10 minutes, rinsed thoroughly with water and autoclaved.
3) RNase-free water should be used to prepare the solution.
4) Operators wear disposable masks and gloves, and change gloves frequently during the experiment.
2. Repeated freezing and thawing of the samples should be avoided, otherwise the quality of extracted DNA, RNA and proteins will be affected. Samples can be stored in Buffer RL at -70° C for one month.
3. Add β-mercaptoethanol to Buffer RL before use, add 10 μl of β-mercaptoethanol to 1 ml of Buffer RL. Buffer RL with β-mercaptoethanol can be stored for 1 month at room temperature.
4. Anhydrous ethanol should be added to Buffer RW2, Buffer GW1 and Buffer GW2 according to the instructions on the label of the reagent bottle before first use.
5. Check Buffer RL for crystals or precipitation before use. If there are crystals or precipitation, re-dissolve in a 56° C water bath.
6. All centrifugation steps were carried out using a bench-top centrifuge at room temperature.

Operational Steps:

1. Material handling
1a. Cells in adherent culture should be processed into cell suspension (maximum extraction volume of 107 cells), collect the cells, discard the culture medium, add 600 μl of Buffer RL (check that β-mercaptoethanol has been added prior to use), and blow the cells repeatedly to make them lysed sufficiently.
Note: Be sure to discard the culture solution or it will interfere with lysis and subsequent nucleic acid purification steps.
1b. Take no more than 30 mg of animal tissue, grind it to a fine powder with liquid nitrogen, add 600 μl of Buffer RL (check that β-mercaptoethanol has been added before use), or add 600 μl of Buffer RL (check that β-mercaptoethanol has been added before use), and homogenize it.
Note: homogenization should be adequate or RNA yield will be affected.
2. Centrifuge the solution obtained in the previous step at 12,000 rpm (~13,400 x g) for 3-5 minutes. Carefully add the supernatant to the Spin Columns DM (Spin Columns

DM) that have been loaded into a collection tube and centrifuge at 12,000 rpm for 30-60 seconds to collect the filtrate. Place the Spin Columns DM in a new 2 ml collection tube and allow to stand at room temperature or 4° C until the DNA is extracted. Note: Ensure that there is no liquid remaining on the Spin Columns, and repeat the centrifugation if necessary until all liquid has passed through the membrane of the Spin Columns. Total RNA Extraction

3. Add 1x volume of 70% ethanol (prepared without RNase water) to the filtrate obtained in step 2 and mix well.

4. Add all of the solution from the previous step to the Spin Columns RM in the collection tube, or transfer to the column in stages if the solution cannot all be added at once. centrifuge at 12,000 rpm for 20 seconds and retain the liquid in the collection tube for protein extraction.

5. Place the adsorbent column RM into a new 2 ml collection tube, add 700 μl of Buffer RW1 to the adsorbent column RM, centrifuge the column at 12,000 rpm for 20 seconds, pour off the waste liquid from the collection tube, and place the adsorbent column RM back into the collection tube.

6. Add 500 μl of Buffer RW2 (check that anhydrous ethanol has been added prior to use) to the adsorbent column RM, centrifuge at 12,000 rpm for 20 seconds, pour off the waste liquid from the collection tube, and return the adsorbent column RM to the 2 ml collection tube.

7. Repeat step 6.

8. Centrifuge at 12,000 rpm for 2 minutes and pour off the waste liquid in the collection tube. Leave the adsorption column at room temperature for several minutes to dry thoroughly.

Note: The purpose of this step is to remove residual ethanol from the adsorption column, which can interfere with subsequent enzymatic reactions (digestion, PCR, etc.).

9. Place the adsorption column RM in a new RNase-free 1.5 ml centrifuge tube, add 30-50 μl of RNase-Free Water to the middle of the adsorption column RM, let it stand at room temperature for 2-5 minutes, centrifuge it at 12,000 rpm for 1 minute, collect the RNA solution, and store the RNA at -70° C to prevent degradation.

Attention:

(1) The volume of RNase-Free Wate should not be less than 30 μl, too small a volume affects the recovery rate.

2) If you want to increase the RNA yield, repeat step 9 with 30-50 μl of fresh RNase-Free Water.

3) If the RNA concentration is to be increased, the resulting solution can be reintroduced into the adsorption column and step 9 repeated.

Genomic DNA extraction

10. Add 500 μl of Buffer GW1 to the adsorption column DM (check that anhydrous ethanol has been added before use), centrifuge at 12,000 rpm for 20 seconds, pour off the waste liquid in the collection tube, and put the adsorption column DM back into the collection tube.

11. Add 500 μl of Buffer GW2 to the adsorbent column DM (check that anhydrous ethanol has been added before use), centrifuge at 12,000 rpm for 2 minutes, pour off the waste liquid in the collection tube, and put the adsorbent column DM back into the collection tube.
Note: Step 11 can be repeated if further DNA purity is required.
12. Centrifuge at 12,000 rpm for 2 minutes and pour off the waste liquid in the collection tube. Leave the adsorbent column DM at room temperature for a few minutes to thoroughly dry out the ethanol in the column.
Note: The purpose of this step is to remove residual ethanol from the adsorption column, which can interfere with subsequent enzymatic reactions (digestion, PCR, etc.).
13. Place the adsorbent column DM in a new centrifuge tube, add 100 μl Buffer GE to the center of the adsorbent column DM overhanging the center of the adsorbent column, leave it at room temperature for 2-5 minutes, centrifuge it at 12,000 rpm for 2 minutes, collect the DNA solution, and store the DNA at -20℃.
Attention:
(1) The volume of Buffer GE should not be less than 100 μl, too small a volume affects the recovery.
2) If you want to increase the yield of DNA, add 100 μl of new Buffer GE to the adsorption column and repeat step 13; if you want to increase the concentration of DNA, you can re-add the DNA eluate obtained in step 13 to the adsorption column and repeat step 13.
protein extraction
14. Add 1 times the volume of Buffer PZ to the RNA extraction effluent (i.e., the solution obtained in step 4), mix thoroughly, and leave at room temperature for 10-30 minutes.
15. Centrifuge at 12,000 rpm for 10 minutes and discard the supernatant.
16. Add 500 μl of 70% ethanol and centrifuge at 12,000 rpm for 1 minute, aspirating the supernatant as much as possible.
17. Leave the centrifuge tube at room temperature for several minutes to dry out the precipitate.
Note: The purpose of this step is to remove the residual ethanol. Excessive drying will make the protein precipitate difficult to dissolve, and incomplete drying of the residual ethanol will affect the protein sample.
18. Add 100 μl Buffer PLS to obtain the protein solution.
Attention:
(1) Protein samples solubilized by Buffer PLS are suitable for SDS-PAGE and Western Blot, but not for protein quantification by the Bradford method. For protein quantification by the Bradford method, 5% SDS can be used to solubilize the protein, or a suitable protein solubilization buffer can be selected according to the downstream experiments.
2) The amount of solubilized protein buffer added is determined based on the initial sample volume and the specific requirements of the downstream test.

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

Protein samples for SDS-PAGE electrophoresis can be performed as follows:

19. Protein Loading Buffer was added to the protein sample, denatured at 95° C for 5-10 minutes, and the sample was cooled to room temperature. 20. Centrifuge at 12,000 rpm for 1 min, and aspirate the supernatant for downstream SDS-PAGE or Western blot etc.