Version No. FRL1903

FineProtect Universal RNA Extraction Kit

(Spin Column)

Catalog No.: R203

Kit Content:

Content	R203 (50 preps)
DNase I (2,000 U)	1 bottle
DNase I Buffer	4 ml
RNase Free ddH_2O (tube)	1 ml
1ml RNase Free syringe	1 pcs
Proteinase K	1.2 ml
RNAstore Reagent	100 ml
Buffer RBL	30 ml
Buffer RLT	40 ml
Buffer RW	40 ml
Buffer RW2	12 ml
RNase Free ddH2O	15 ml
RNAPure Spin Columns	50 pcs
2 ml Collection Tubes	50 pcs
1.5 ml RNase Free Microcentrifuge Tubes	50 pcs

Storage：

DNase I DNase I Buffer and RNase Free ddH2O are transported in ice bags and immediately stored at 2-8℃ upon receipt. Buffer RLT with β-mercaptoethanol can be stored at 4℃ for up to 1 month, and the other reagents are stored at room temperature (15-25℃).

This Kit Special for Scientific Research.

Introduction

This kit provides a solution combination for RNA preservation to extraction. RNA store Reagent is a non-toxic tissue preservation solution which can rapidly infiltrates into tissues to protect RNA. By inhibiting RNase activity, it can protect in situ RNA in non-frozen cell. The tissues samples are more suitable for gene expression analysis. The samples in this preservation reagent can be stored for a long time, and even if subjected to repeated frozen-thawed, RNA in the preserved samples will not degrade. The preserved samples can be stored at 37°C for 1 day, 18-25°C for 7 days, and 2-8°C for 4 weeks; and moreover samples can be treated, stored, and transported at room temperature. The samples protected by this reagent can be kept at -20°C or -80°C for a long-term storage. The tissues samples in this preservation solution can be ditectly used for RNA purification. The unique lysis buffer adopted by the kit is efficient, fast and convenient and does not require the extraction of phenol and chloroform in the RNA isolation process. It is suitable for extraction of the total RNA from the animal tissues, animal cells or bacteria. This kit can be used to effectively extract RNA fragments greater than 200 nt in size. The RNA extracted with this kit is of high purity and is rarely contaminated by proteins, genomic DNA and other impurities. The extracted RNA can be directly used for Northern hybridization, dot hybridization, mRNA purification, in vitro translation, RNA protection analysis, RT-PCR, RT-qPCR, cDNA library construction and other molecular biology experiments.

Precautions:

Be sure to read the precautions before using this kit.

1. Before operation, β-mercaptoethanol is added to Buffer RLT to the final concentration of 1%. The prepared RLT can be stored at 4°C for up to 1 month. Buffer RLT may form precipitates during storage. If precipitates appear, please heat and dissolve them at 60°C and use them after restoring to room temperature.
2. Whenever possible, use fresh collected samples. RNA yield is dependent on the integrity of the RNA in the initial sample, and RNA that has been degraded into small fragments according to the standard procedures in this manual cannot be effectively recovered.
3. To extract the RNA containing Small RNA (<200 nt), ask GENFINE for the corresponding extraction process.

Preparation of DNase I stock solution:

Absorb 550 µl RNase Free ddH2O with 1 ml syringe firstly, inject it into a glass bottle containing DNase I dry powder (2,000 U), mix gently, dispense and store at -20°C (it can be stored for 9 months).

Note: DNase I stock solution melt at -20℃ is stored at 4℃ (can be stored for 6 weeks) and shall not be cryopreserved again.

Sample preservation steps:

Preserve fresh tissue samples

1. Quickly cut the fresh tissues to a thickness of less than 0.5 cm and place in the RNase Free collection tube prefilled with the RNAstore Reagent (1 ml RNAstore Reagent is required for every 100 mg of tissue).
2. The collection tube is stored under appropriate conditions. The protected RNA can be stored at 37°C for 1 day, 18-25°C for 7 days, and 2-8°C for 4 weeks, and samples can be operated, stored, and transported free of dry ice or liquid nitrogen. The samples protected by this reagent can be stored at -20℃ or -80℃ for a long time. If stored at -20℃ or -80℃, the samples shall be placed at 4℃ overnight, centrifuged to discard the protective solution, and then transferred to -20℃ or -80℃.
3. RNA extraction: take out the preserved tissue samples. The samples stored at -20℃ or -80℃ and shall be melted at room temperature first.
4. Immediately extract the total RNA from the tissues according to this manual.

Preserve culture cells, suspension cells and bacteria

1. Sample treatment:

   1.1 For tissue culture cell samples: first precipitate cells, wash them once with PBS, suspend cells with a small amount of PBS, and then add 2 times volume of RNA store Reagent for storage. Subsequently, RNA may be extracted after removal of RNA store Reagent by direct centrifugation or extracted directly by directly adding RNA extraction lysate without removing RNA store Reagent.

   a. Centrifugation method: Since the concentration of the RNA store Reagent is higher than that of typical cell culture media, the cells in the RNA store Reagent cannot be precipitated by the centrifugal force that normally precipitates living cells. Centrifuge and precipitate the cells to remove RNA store Reagent. (About 3000×g is required for HeLa cells, but other cells may not tolerate this speed, or they may need greater centrifugal force.)

   b. Direct extraction method: add 10 times volume of RNA extraction reagent to the cell mixture.

   1.2 For bacteria samples: first centrifuge and collect bacteria, wash them once with PBS, suspend cells with a small amount of PBS, and then add 2 times volume of RNA store Reagent for storage. RNA is extracted

subsequently according to the extraction steps of total RNA from bacteria. The E.coli can be stored for 1 month in RNA store Reagent at 4℃.

2. The collection tube is stored under appropriate conditions. The protected RNA can be stored at 37°C for 1 day, 18-25°C for 7 days, and 2-8°C for 4 weeks, and samples can be operated, stored, and transported free of dry ice or liquid nitrogen. The samples protected by this reagent can be stored at -20℃ or -80℃ for a long time. If stored at -20℃ or -80℃, the samples shall be placed at 4℃ overnight, centrifuged to discard the protective solution, and then transferred to -20℃ or -80℃.
3. RNA extraction: take out the preserved cell or bacteria samples. The samples stored at -20℃ or -80℃ shall be melted at room temperature first.
4. Immediately extract the total RNA from the bacteria or cells according to this manual.

Total RNA extraction steps:

Before use, add anhydrous ethanol to Buffer RW2 in the volume specified on the bottle.

Extract total RNA from tissues

1. Column equilibration steps: add 500 μL Buffer RBL to the adsorption column RNA Pure Spin Columns **(put the adsorption column into the collection tube)**, centrifuge at 12,000 rpm for 2 min, dump the flow-through in the collection tube, and put the adsorption column back into the collection tube. **(Use the column treated on the same day)**
2. Homogenization:

   Quickly transfer the RNA store Reagent preserved or fresh tissue samples to a mortar precooled with liquid nitrogen, grind the tissues with a pestle and add liquid nitrogen continuously until the tissues are ground to powder **(no obvious visible particles. If the tissues are not ground completely, the yield and quality of RNA will be affected)**. Add the ground powder sample (10-20 mg) into a 1.5 mL sterile centrifuge tube containing 300 μL Buffer RLT (**check whether β-mercaptoethanol has been added before use**), bring to vortex, shake vigorously and mix until there is no obvious precipitation in the lysate. Then add 590 μl RNase Free ddH_2O and 10 μl Proteinase K to the tissue homogenate, mix evenly and incubate at 56℃ for 10-20 min.

   Note: Or add 300 μl Buffer RLT to 10-20 mg RNA store Reagent preserved or fresh tissues (check whether β-mercaptoethanol has been added before use) and grind thoroughly with electric or glass homogenizer. The tissue amount must not exceed 20 mg, otherwise the yield and purity of RNA will

decrease.

3. Centrifuge at 12,000 rpm for 2 min and transfer the supernatant to another centrifuge tube.
4. Slowly add 0.5 times filtrate volume of anhydrous ethanol, mix well (precipitation may appear at this point), transfer the solution and precipitation obtained to the adsorption column FineBind DNA Spin Columns (the adsorption column is placed in the collection tube), centrifuge at 12,000 rpm for 30 sec, discard the waste liquid in the collection tube and put the adsorption column back in the collection tube.
5. Add 350 µl Buffer RW to the adsorption column, centrifuge at 12,000 rpm for 30 sec, discard the waste liquid in the collection tube and put the adsorption column back in the collection tube.
6. Preparation of DNase I working solution: add 10 µl DNase I stock solution to a new RNase Free centrifuge tube, add 70 µl DNase I Buffer and mix gently. (Preparation of DNase I stock solution: dissolve DNase I dry powder (2,000 U) in 550 µl RNase Free ddH_2O, mix gently, dispense and store at -20℃ (it can be stored for 9 months).)

   Note: The thawed DNase I is stored at 4℃ (it can be stored for 6 weeks) and shall avoid repeated freezing and thawing.
7. Add 80 µl DNase I working solution to the center of the adsorption column and place at room temperature for 15 min.
8. Add 350 µl Buffer RW to the adsorption column, centrifuge at 12,000 rpm for 30 sec, discard the waste liquid in the collection tube and put the adsorption column back in the collection tube.
9. Add 500 µl Buffer RW2 **(check whether ethanol has been added before use)** to the adsorption column, centrifuge at 12,000 rpm for 30 sec, discard the waste liquid in the collection tube and put the adsorption column back in the collection tube.
10. Repeat step 9 once.
11. Centrifuge at 12,000 rpm for 3 min, put the adsorption column in a new 1.5 ml RNase Free
12. Microcentrifuge Tubes, add 30-100 µl RNase Free ddH2O to the center of the adsorption film,
13. and centrifuge at 12,000 rpm for 1 min to obtain RNA solution.

Extract total RNA from culture cells

1. Column equilibration steps: add 500 µL Buffer RBL to the adsorption column RNAPure Spin Columns **(put the adsorption column into the collection tube)**, centrifuge at 12,000 rpm for 2 min, dump the flow-through

in the collection tube, and put the adsorption column back into the collection tube. **(Use the column treated on the same day)**

2. Homogenization:

   Quickly transfer the RNAstore Reagent preserved or fresh tissue samples to a mortar precooled with liquid nitrogen, grind the tissues with a pestle and add liquid nitrogen continuously until the tissues are ground to powder **(no obvious visible particles. If the tissues are not ground completely, the yield and quality of RNA will be affected)**. Add the ground powder sample (10-20 mg) into a 1.5 mL sterile centrifuge tube containing 300 µL Buffer RLT (**check whether β-mercaptoethanol has been added before use**), bring to vortex, shake vigorously and mix until there is no obvious precipitation in the lysate. Then add 590 µl RNase Free ddH_2O and 10 µl Proteinase K to the tissue homogenate, mix evenly and incubate at 56℃ for 10-20 min.

   Note: Or add 300 µl Buffer RLT to 10-20 mg RNAstore Reagent preserved or fresh tissues (check whether β-mercaptoethanol has been added before use) and grind thoroughly with electric or glass homogenizer. The tissue amount must not exceed 20 mg, otherwise the yield and purity of RNA will decrease.

3. Centrifuge at 12,000 rpm for 2 min and transfer the supernatant to another centrifuge tube.
4. Slowly add 0.5 times filtrate volume of anhydrous ethanol, mix well **(precipitation may appear at this point)**, transfer the solution and precipitation obtained to the adsorption column FineBind DNA Spin Columns **(the adsorption column is placed in the collection tube)**, centrifuge at 12,000 rpm for 30 sec, discard the waste liquid in the collection tube and put the adsorption column back in the collection tube.
5. Add 350 µl Buffer RW to the adsorption column, centrifuge at 12,000 rpm for 30 sec, discard the waste liquid in the collection tube and put the adsorption column back in the collection tube.
6. Preparation of DNase I working solution: add 10 µl DNase I stock solution to a new RNase Free centrifuge tube, add 70 µl DNase I Buffer and mix gently. (Preparation of DNase I stock solution: dissolve DNase I dry powder (2,000 U) in 550 µl RNase Free ddH2O, mix gently, dispense and store at -20℃ (it can be stored for 9 months).)

   Note: The thawed DNase I is stored at 4℃ (it can be stored for 6 weeks) and shall avoid repeated freezing and thawing.

7. Add 80 µl DNase I working solution to the center of the adsorption column and place at room temperature for 15 min.

8. Add 350 µl Buffer RW to the adsorption column, centrifuge at 12,000 rpm for 30 sec, discard the waste liquid in the collection tube and put the adsorption column back in the collection tube.
9. Add 500 µl Buffer RW2 (check whether ethanol has been added before use) to the adsorption column, centrifuge at 12,000 rpm for 30 sec, discard the waste liquid in the collection tube and put the adsorption column back in the collection tube.
10. Repeat step 9 once.
11. Centrifuge at 12,000 rpm for 3 min, put the adsorption column in a new 1.5 ml RNase Free Microcentrifuge Tubes, add 30-100 µl RNase Free ddH2O to the center of the adsorption film, and centrifuge at 12,000 rpm for 1 min to obtain RNA solution.

Extract total RNA from culture cells

1. Column equilibration steps: add 500 µL Buffer RBL to the adsorption column RNAPure Spin Columns (put the adsorption column into the collection tube), centrifuge at 12,000 rpm for 2 min, dump the waste liquid in the collection tube, and put the adsorption column back into the collection tube. (Use the column treated on the same day)
2. Homogenization

When RNA store Reagent is not used:

Adherent cell: absorb the medium thoroughly, add 600 µl Buffer RLT to every 6-10 cm^2 of area (**check whether β-mercaptoethanol has been added before use**) and use a pipette to blow 3-5 times to lyse the cells.

Cell suspension: centrifuge and collect cells at 500 x g, add 600 µl Buffer RLT to every 5×10^6-1×10^7 cells (**check whether β-mercaptoethanol has been added before use**), add 350 µl Buffer RLT to the cells fewer than 5×10^6 (**check whether β-mercaptoethanol has been added before use**)and use a pipette to blow 3-5 times to lyse the cells.

When RNA store Reagent is used:

Add 600 µl Buffer RLT to every 1×106-1×107 cells collected by RNAstore Reagent **(check whether β-mercaptoethanol has been added before use)**, add 350 µl Buffer RLT to the cells fewer than 5×106 **(check whether β-mercaptoethanol has been added before use)** and use a pipette to blow 3-5 times to lyse the cells.

3. **(Optional)** Transfer all solutions to FinePure Filtration Columns **(self-provided by the customer, purchased from GENFINE and placed in the collection tube)**, centrifuge at 12,000 rpm for 2 min and carefully pipette the filtrate in the collection tube to a new 1.5 ml RNase Free centrifuge tube. The sucker shall not contact with the cell debris precipitation in the collection tube.
4. Slowly add 0.5 times filtrate volume of anhydrous ethanol, mix well **(precipitation may appear at this point)**, transfer the solution and precipitation obtained to the adsorption column FineBind DNA Spin Columns **(the adsorption column is placed in the collection tube)**, centrifuge at 12,000 rpm for 30 sec, discard the waste liquid in the collection tube and put the adsorption column back in the collection tube.
5. Add 350 µl Buffer RW to the adsorption column, centrifuge at 12,000 rpm for 30 sec, discard the waste liquid in the collection tube and put the adsorption column back in the collection tube.
6. Preparation of DNase I working solution: add 10 µl DNase I stock solution to a new RNase Free centrifuge tube, add 70 µl DNase I Buffer and mix gently. (Preparation of DNase I stock solution: dissolve DNase I dry powder (2,000 U) in 550 µl RNase Free ddH2O, mix gently, dispense and store at -20℃ (it can be stored for 9 months).)

   Note: The thawed DNase I is stored at 4℃ (it can be stored for 6 weeks) and shall avoid repeated freezing and thawing.
7. Add 80 µl DNase I working solution to the center of the adsorption column and place at room temperature for 15 min.
8. Add 350 µl Buffer RW to the adsorption column, centrifuge at 12,000 rpm for 30 sec, discard the waste liquid in the collection tube and put the adsorption column back in the collection tube.
9. Add 500 µl Buffer RW2 **(check whether ethanol has been added before use)** to the adsorption column, centrifuge at 12,000 rpm for 30 sec, discard the waste liquid in the collection tube and put the adsorption column back in the collection tube.
10. Repeat step 9 once.
11. Centrifuge at 12,000 rpm for 3 min, put the adsorption column in a new 1.5 ml RNase Free Microcentrifuge Tubes, add 30-100 µl RNase Free ddH_2O to the center of the adsorption film,
12. and centrifuge at 12,000 rpm for 1 min to obtain RNA solution.

Extract total RNA from bacteria

1. Column equilibration steps: add 500 μL Buffer RBL to the adsorption column RNAPure Spin Columns **(put the adsorption column into the collection tube)**, centrifuge at 12,000 rpm for 2 min, dump the waste liquid in the collection tube, and put the adsorption column back into the collection tube. **(Use the column treated on the same day)**
2. Collect the bacteria (not exceeding 1×10^9) according to the usage method of RNAstore Reagent in this manual and carefully remove the supernatant.
3. Thoroughly resuspend the bacteria with 100 μl TB buffer containing Lysozyme and incubate at room temperature. The usage and incubation time of Lysozyme are shown in the following table.

Bacteria type	Final concentration of Lysozyme in TB buffer	Incubation time
G- Bacteria	400 μg/ml	3-5 min
G+ Bacteria	3 mg/ml	5-10 min

4. Add 350μl Buffer RLT (**check whether β-mercaptoethanol has been added before use**), bring to vortex shaking and mixing **(precipitate may appear at this step)**, shake and mix. If insoluble precipitate appears, centrifuge at 12,000 rpm for 2 min and transfer the supernatant to another centrifuge tube.
5. Slowly add 0.5 times filtrate volume of anhydrous ethanol, mix well **(precipitation may appear at this point)**, transfer the solution and precipitation obtained to the adsorption column FineBind DNA Spin Columns **(the adsorption column is placed in the collection tube)**, centrifuge at 12,000 rpm for 30 sec, discard the waste liquid in the collection tube and put the adsorption column back in the collection tube.
6. Add 350 μl Buffer RW to the adsorption column, centrifuge at 12,000 rpm for 30 sec, discard the waste liquid in the collection tube and put the adsorption column back in the collection tube.
7. Preparation of DNase I working solution: add 10 μl DNase I stock solution to a new RNase Free centrifuge tube, add 70 μl DNase I Buffer and mix gently. (Preparation of DNase I stock solution: dissolve DNase I dry powder (2,000 U) in 550 μl RNase Free ddH_2O, mix gently, dispense and store at -20℃ (it can be stored for 9 months).)

   Note: The thawed DNase I is stored at 4℃ (it can be stored for 6 weeks) and shall avoid repeated freezing and thawing.
8. Add 80 μl DNase I working solution to the center of the adsorption column and place at room temperature for 15 min.

9. Add 350 µl Buffer RW to the adsorption column, centrifuge at 12,000 rpm for 30 sec, discard the waste liquid in the collection tube and put the adsorption column back in the collection tube.
10. Add 500 µl Buffer RW2 (**check whether ethanol has been added before use**) to the adsorption column, centrifuge at 12,000 rpm for 30 sec, discard the waste liquid in the collection tube and put the adsorption column back in the collection tube.
11. Repeat step 10 once.
12. Centrifuge at 12,000 rpm for 3 min, put the adsorption column in a new 1.5 ml RNase Free Microcentrifuge Tubes, add 30-100 µl RNase Free ddH_2O to the center of the adsorption film, and centrifuge at 12,000 rpm for 1 min to obtain RNA solution.

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