TIANGEN®

Magnetic Tissue/ Cell/Blood Total RNA Kit

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Magnetic Tissue/Cell/Blood Total RNA Kit

Cat.no. GDP761

Kit Contents

Contents	GDP761 (50 preps)
Buffer RL	30 ml
Buffer RD	48 ml
Buffer RW	2×12 ml
RNase-Free ddH_2O	15 ml
MagAttract Suspension W	2×1 ml
Handbook	1

Optional reagents and tools

DNase I (Cat.no. GRT411); Magnetic Stand (Cat.no. OSE-MF-01); 10×Red cell Lysis Buffer H; RNase-Free Columns CS set.

Storage

This kit can be stored at room temperature (15-30°C) under dry condition for 15 months.

Introduction

The kit adopts magnetic beads with unique separation function and a unique buffer system to separate and purify high-quality total RNA from various tissues, cells and blood. The unique embedded magnetic beads have strong affinity for nucleic acid under certain conditions, and when the conditions change, the magnetic beads will release adsorbed nucleic acid, thus achieving the purpose of fast separation and purification of nucleic acid.

The product can be perfectly matched with Kingfisher Flex96 and TGuide S32 Automated Nucleic Acid Extractors. Magnetic beads are adsorbed, transferred and released by special magnetic rods, thus realizing the transfer of magnetic beads and nucleic acids. The whole experimental process is safe and convenient, and the extracted total RNA has high purity and is free from contamination of genomes, proteins and other impurities. If high-throughput automated extraction is required, please contact TIANGEN for the integration solution.

RNA purified by the kit is suitable for various downstream experiments such as RT-PCR, Real Time RT-PCR, chip analysis, Northern Blot, Dot Blot, PolyA screening, *in vitro* translation, RNase protection analysis, molecular cloning, etc.

Features

1. The kit can meet the requirements of manual extraction as well as batch extraction on various high-throughput platforms.
2. The products obtained by the kit meet the requirements of downstream detection experiments and NGS analysis.

Notes <u>Please read these notes before using this kit.</u>

1. Before operation, add β-mercaptoethanol to Buffer RL to a final concentration of 1%, for example, add 10 µl of β-mercaptoethanol to 1 ml of Buffer RL. This buffer is best prepared right before use. The prepared Buffer RL can be stored at 2-8°C for 1 month. The Buffer RL may form precipitates during storage, and if any precipitates appear, please heat and dissolve before use.
2. Add 96-100% ethanol to Buffer RD and Buffer RW before the first use. Please refer to the label on the bottle for the added volume.

Self-provided reagents and instruments

β-mercaptoethanol, isopropanol, 96-100% ethanol, homogenization equipment (mortar, electric homogenizer, etc.), magnetic stand.

I. Tissue/cell total RNA extraction

1. Sample treatment

1) Tissue sample homogenate:

Note: The amount of tissue should not exceed 20 mg, otherwise the yield and quality of RNA may decrease. For DNA-rich samples, such as spleen, 5 mg is recommended.

Take out fresh or frozen tissue at -80°C and grind it into powder with liquid nitrogen. Weigh 5-20 mg to 1.5 ml centrifuge tube containing 450 µl of Buffer RL (or use an electric homogenizer for the grinding) **(ensure β-mercaptoethanol is added before use)**. Vortex and mix immediately, let it stand at room temperature for 5 min, centrifuge at 12,000 rpm for 5 min, and carefully remove the supernatant.

2) Cell sample treatment:

a.Collection of suspended cells (the number of collected cells should not exceed 1×10^7): Estimate the number of cells, centrifuge at 300×g for 5 min, collect the cells into a centrifuge tube, and carefully remove all the supernatant of the culture medium.

b.Trypsin treatment method: Determine the number of cells, then remove the culture medium, and wash the cells with PBS solution. Dispose the PBS solution, add PBS solution containing 0.10-0.25% trypsin to the cells to treat the cells, when the cells are separated from the container wall, add the culture medium containing serum to inactivate trypsin. Transfer the cell solution to aRNase-Free centrifuge tube, centrifuge for 5 min at 300×g to collect the cell precipitate, and carefully remove all supernatant.

Note: When collecting cells, the cell culture solution must be removed completely, otherwise it will lead to incomplete lysis, affect the binding of RNA and magnetic beads, and reduce the yield of RNA.

Cell lysis

No. of cells	Buffer RL (µl)
$<5\times10^6$	450
5×10^6-1×10^7	600

2. Take 450 µl of the above sample homogenate, add 400 µl of isopropanol and 40 µl of MagAttract Suspension W, and mix the magnetic beads evenly for 5 min by an oscillator to bind RNA.
3. Place the centrifuge tube on a magnetic stand and let it stand for 3 min. After the magnetic beads are completely attached, carefully dispose the liquid.
4. Remove the centrifuge tube from the magnetic stand, add 900 µl of Buffer RD **(ensure that 96-100% ethanol is added before use)**, and mix well for 2 min.
5. Place the centrifuge tube on a magnetic stand and let it stand for 3 min. After the magnetic beads are completely attached, carefully remove the liquid and discard it. Dry it at room temperature for 5 min.
6. **(Optional)** Remove the centrifuge tube from the magnetic stand, and perform DNase I digestion:

   Add 5 µl DNase I into 70 µl Buffer RDD to make the DNase I working solution. Add the prepared working solution into the sample tube, and place it at room temperature for 15 min, during which mix it evenly every 5 min.
7. Add 700 µl of Buffer RD **(ensure that 96-100% ethanol is added before use)** and mix with oscillator for 5 min.
8. Place the centrifuge tube on a magnetic stand and let it stand for 3 min. After the magnetic beads are completely attached, carefully remove the liquid and discard it.
9. Remove the centrifuge tube from the magnetic stand, add 700 µl of Buffer RW **(ensure that 96-100% ethanol is added before use)**, and mix evenly with the oscillator for 2 min.
10. Place the centrifuge tube on the magnetic stand and let it stand for 3 min. After the magnetic beads are completely attached, carefully remove the liquid and discard it.
11. Repeat steps 9 and 10 once.
12. Collect the residual solution to the bottom of the tube by short centrifugation. Place the centrifuge tube on a magnetic stand, carefully remove all the liquid, and dry at room temperature for 5 min.
13. Remove the centrifuge tube from the magnetic stand, add 50-100 µl RNase-Free ddH_2O, heat and elute at 60°C for 5 min, and mix it by inverting up and down for 4-5 times to fully elute RNA (or place it on a heating oscillator for elution).
14. Place the centrifuge tube on the magnetic stand and let stand for 3 min. After the magnetic beads are completely attached, carefully transfer the RNA solution to a clean centrifuge tube and continue the subsequent experiment or store it at -80°C.

II. Blood total RNA extraction

Note: This kit is only suitable for extracting total RNA from fresh blood, not frozen blood.

1. Dilution of 10×Red cell Lysis Buffer H: Choose an appropriate volume of 10×Red cell Lysis Buffer H (self-provided) according to the volume of the treated blood sample (for example, take 140 µl of 10×Red cell Lysis Buffer H if the volume of the blood sample to be treated is 200 µl), and dilute to 1×Red cell Lysis Buffer H with RNase-Free ddH_2O.
2. Add 5 volumes of 1×Red cell Lysis Buffer H to 1 volume of fresh whole blood (proper clean tubes should be provided).

   Notes:

   a. In order to obtain the best mixing effect, the volume of the mixed solution of blood and 1× Red cell Lysis Buffer H should not exceed 3/4 of the tube volume. If the white blood cell content in blood is high, the volume of blood used can be reduced proportionally, and the volume of Buffer RL used in step 7 should be adjusted accordingly.

   b. Fresh whole blood here refers only to mammals. Since the red blood cells of poultry contain nucleic acid, this step is not required, and the initial amount of blood can be determined according to the requirements to directly perform whole blood lysis (i.e. step 7).
3. Incubate on ice for 10-15 min, and mix evenly for 2 times by vortex during incubation.

   Note: During incubation, the solution will become translucent, indicating lysis of red blood cells. If necessary, the incubation time can be extended to 20 min.
4. Centrifuge at 2,100 rpm (~400×g) for 10 min at 2-8°C to completely remove the supernatant.

   Note: White blood cells may form globules after centrifugation, please ensure complete removal of supernatant. The presence of trace amount of red blood cells will make white blood cell globules appear red, and this phenomenon will disappear in the subsequent washing step.
5. Add 1×Red cell Lysis Buffer H (the volume of 1×Red cell Lysis Buffer H added is 2 times of the total blood in step 1) to leukocyte precipitation to resuspend cells.
6. Centrifuge at 2-8°C, 2,100 rpm (~400×g) for 10 min to completely remove the supernatant.

7. Add Buffer RL to leukocyte precipitation **(ensure that β-mercaptoethanol is added before use)**. The specific dosage is as follows, vortex or mix evenly with a pipette.

   Note: If the blood is not the whole blood of a healthy person, the volume of Buffer RL should be determined according to the number of white blood cells in the blood. At this step, the cells should be completely lysed and the massive cell precipitation will disappear.

Buffer RL(μl)	Healthy human whole blood (ml)	No. of white blood cell
400	Up to 0.5	Up to 2×10^6
600	0.5-1.5	2×10^6 to 1×10^7

8. **(Optional)** Transfer the solution to the Column CS (the Column CS is placed in the collection tube), centrifuge at 12,000 rpm (~ 13,400 x g) for 2 min, discard the Column CS, and collect the filtrate.

   Note: This step is recommended when there are coagulation spots or tablets, otherwise it will affect the binding of nucleic acids and magnetic beads and the subsequent purification.

9. Add 1 volume of isopropanol (usually 400 μl or 600 μl) to the above filtrate and 40 μl of MagAttract Suspension W, mix evenly with the oscillator for 5 min, and allow the magnetic beads to bind RNA.
10. Place the centrifuge tube on a magnetic stand and let it stand for 3 min. After the magnetic beads are completely attached, carefully remove the liquid and discard it.
11. Remove the centrifuge tube from the magnetic stand, add 900 μl of Buffer RD **(ensure that ethanol is added before use)**, and mix well for 2 min.
12. Place the centrifuge tube on a magnetic stand and let it stand for 3 min. After the magnetic beads are completely attached, carefully remove the liquid and discard it. Dry it at room temperature for 5 min.
13. **(Optional)** Remove the centrifuge tube from the magnetic stand for DNase I digestion:

    Add 5 μl DNase I into 70 μl RDD buffer to make the DNase I working solution. Add the prepared working solution into the sample tube, and mix it by inverting up and down every 5 min at room temperature for 15 min. (Or use an oscillator to oscillate for 15 min).
14. Add 700 μl of Buffer RD **(ensure that ethanol is added before use)** and mix well with oscillator for 5 min.

15. Place the centrifuge tube on a magnetic stand and let it stand for 3 min. After the magnetic beads are completely attached, carefully remove the liquid and discard it.
16. Remove the centrifuge tube from the magnetic stand, add 700 µl of Buffer RW **(ensure that ethanol is added before use)**, and mix evenly with the oscillator for 2 min.
17. Place the centrifuge tube on a magnetic stand and let it stand for 3 min. After the magnetic beads are completely attached, carefully remove the liquid and discard it.
18. Repeat step 16 and 17 once.
19. Collect the residual solution to the bottom of the tube by short centrifugation. Place the centrifuge tube on a magnetic stand, remove and discard all the liquid, and dry at room temperature for 5 min.
20. Remove the centrifuge tube from the magnetic stand, add 50-100 µl RNase-Free ddH_2O, heat and elute at 60°C for 5 min, and mix it by inverting up and down for 4-5 times to fully elute RNA (or place it on a heating oscillator for elution).
21. Place the centrifuge tube on a magnetic rack and let it stand for 3 min. After the magnetic beads are completely adsorbed, carefully transfer the RNA solution to a clean centrifuge tube and continue the subsequent experiment or keep it at -80°C.