RNAprep Pure Hi-Blood Kit

For purification of high-quality and stable total RNA from blood

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Cat.no. GDP443

Kit Contents

Contents		GDP443 (50 preps)
GDP443H	10 × Red Cell Lysis Buffer H	60 ml
	Buffer RLH	30 ml
	Buffer RW1H	24 ml
	Buffer RW	12 ml
	RNase-Free ddH_2O	15 ml
	RNase-Free Columns CR4 set	50
	RNase-Free Columns CS set	50
	RNase-Free Centrifuge Tubes (1.5 ml)	50
	Handbook	1
GRT411	RNase-Free DNase I (1500 U)	1
	Buffer RDD (DNA Digest Buffer)	4 ml
	RNase-Free ddH_2O	1 ml

Note: GDP443H, GRT411 are shipped and packaged separatly.

Storage

The Buffer RLH added with β-mercaptoethanol can be kept at 2-8°C for one month. DNase I and Buffer RDD should be stored at 2-8°C for 15 months. Other reagents should be stored at room temperature (15-30°C) for 15 months.

Introduction

The kit can efficiently extract total RNA from fresh whole blood and blood with various anticoagulants of different species. The silica matrix material used in the adsorption column is a unique new material of TIANGEN, which can adsorb RNA efficiently and specifically and can remove protein impurities to the maximum extent. The extracted RNA can be used in RT-PCR, Real Time RT-PCR, chip analysis, high-throughput sequencing, Northern blot, Dot blot, Poly A screening, *in vitro* translation, RNase protection analysis, molecular cloning , etc.

Please Pay Attention to The Following Aspect In Order to Prevent RNase Pollution

1. Change new gloves frequently. Because the skin is often contaminated with bacteria, it may contaminate RNase.
2. Use RNase-free plastic products and pipette tip to avoid cross contamination.
3. RNA will not be degraded by RNase in Buffer RLH. However, RNase-free plastic and glassware should be used in the further processing after extraction. Bake glassware at 150°C for 4 h, or soak plastic ware in 0.5 M NaOH for 10 min, then thoroughly wash with water and autoclave to remove RNase.
4. RNase-free ddH_2O should be used to prepare the solution. (Add water into a clean glass bottle, add DEPC to a final concentration of 0.1% (V/V), mix well and then place overnight, sterilize with autoclave.)

Important Notes Please read these notes before using this kit.

1. Before operation, add β-mercaptoethanol to Buffer RLH to a final concentration of 1%. (For example, add 10 μl β- mercaptoethanol to 1 ml RLH). It's better to prepare the buffer when using. The prepared RLH can be stored at 2-8°C for one month. The precipitate might formed in Buffer RLH during storage.If there is precipitate, please use it after heating and dissolving.
2. Add 96-100% ethanol to Buffer RW and Buffer RW1H before the first time using. Please refer to the label on the bottle for the added volume.
3. Human blood or body fluid may have potential infectivity, so please pay attention to take protective measures when processing human whole blood.
4. The kit can process up to 1.5 ml of healthy human whole blood (the leukocyte content in the whole blood of healthy people is up to 4000-7000 cells/μl blood). If the leukocyte content in the blood is high, the amount of blood can be reduced proportionally, and the maximum number of leukocyte that can be processed by the kit is 1×10^7.
5. After leukocyte lysis, all the steps in this manual should be carried out at room temperature (15-30°C), and please perform all steps as fast as possible.
6. Cell lysate (in Buffer RLH) can be stored at -70°C When ready to use, incubate it at 37°C for 10 minutes to ensure that all salts are dissolved, then proceed to step 7 of the procedure.
7. This kit is not suitable for cryopreserved whole blood.

Preparation of DNase I storage solution

Dissolve DNase I dry powder (1500 U) in 550 µl RNase-Free ddH_2O, mix gently, and store at -30~-15°C after aliquoting (it can be stored for 9 months). (If DNase I is required, please enquire TIANGEN)

Note: DNase I storage solution thawed from -30~-15°C shall be stored at 2-8°C (can be stored for 6 weeks) and shall not be frozen again.

Protocol

1. Dilution of Red Cell Lysis Buffer H: Select an appropriate volume of 10× Red Cell Lysis Buffer H 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 processing blood sample is 200 µl), and dilute to 1× Red Cell Lysis Buffer H with RNase-Free ddH_2O.
2. Add 5 times the volume of 1× Red Cell Lysis Buffer H to 1 volume of human whole blood (proper clean tube should be self-provided).

   Note: 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 leukocyte content in blood is high, the volume of blood used can be reduced proportionally, and the volume of 1× Red Cell Lysis Buffer H used in step 6 should be adjusted accordingly.
3. Incubate on ice for 10-15 min, and mix evenly for 2 times by vortex during incubation.

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

   Note: Leukocyte may form globules after centrifugation. Make sure to completely remove the supernatant. The presence of trace red blood cells will make leukocyte globules appear red, and this phenomenon will disappear in the subsequent washing step.
5. Add 1 × Red Cell Lysis Buffer H (the added volume of 1× Red Cell Lysis Buffer H is 2 times of the total blood consumption in the step 1) to leukocyte precipitation, and resuspend cells.

6. Centrifuge at 2,100 rpm (~400 × g) for 10 min at 4°C to completely remove the supernatant.

   Note: Incomplete removal of supernatant will affect lysis and the subsequent binding of RNA to membrane, resulting in reduction of final RNA yield.

7. Add Buffer RLH **(please add β-mercaptoethanol before use)** to leukocyte precipitation. 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 RLH should be determined according to the number of leukocyte in the blood. At this time, the cells should be completely lysed and the massive cell precipitation will disappear.

Buffer RLH (μl)	Healthy human whole blood (ml)	Leukocyte amount
350	Up to 0.5	Up to 2×10^6
600	0.5-1.5	2×10^6 to 1×10^7

8. Transfer the solution to the RNase-Free Column CS (place the Column CS in a collection tube) and centrifuge at 12,000 rpm (~ 13,400 × g) for 2 min, discard the Column CS and collect the filtrate.

   Note: To avoid the formation of aerosol, please adjust the pipette to ≥ 750 ul to ensure that all the solutions are transferred to the filter column at one time. If there are too many cells, the lysis solution will be viscous and difficult to aspirate.

9. Add the same volume of 70% ethanol (usually 350 μl or 600 μl) to the filtrate, mix well (precipitation may occur at this time), transfer the obtained solution and precipitation into RNase-Free Column CR4 (place the Column CR4 in a collection tube), centrifuge at 12,000 rpm (~13,400 × g) for 30-60 sec, discard the waste liquid in the collection tube, and return Column CR4 to the collection tube.

   Note: Use RNase-Free ddH_2O when preparing 70% ethanol. If there is volume lost of filtrate, reduce the dosage of 70% ethanol accordingly. If the solution volume is larger than the capacity of the CR4, so step 9 can be processed twice to collect all the solution.

10. If DNase I digestion is not carried out, 700 μl of Buffer RW1H can be directly added to Column CR4 **(ensure that ethanol has been added before use)**. Centrifuge at 12,000 rpm (~13,400×g) for 30-60 sec, discard waste liquid in the collection tube, and directly proceeded to step 14.

DNase I digestion: Add 350 µl of Buffer RW1H to Column CR4, centrifuge at 12,000 rpm (~13,400 x g) for 30-60 sec, discard waste liquid in the collection tube and put the Column CR4 back into the collection tube.

11. Preparation of DNase I working solution: Transfer 10 µl DNase I storage solution to a new RNase-Free centrifuge tube, add 70 µl RDD solution, and mix gently.
12. Add 80 µl of DNase I working solution to the center of Column CR4 and place at room temperature for 15 min.
13. Add 350 µl Buffer RW1H to Column CR4, centrifuge at 12,000 rpm (~13,400 × g) for 30-60 sec, discard the waste liquid in the collection tube, and return Column CR4 to the collection tube.
14. Add 500 µl of Buffer RW to Column CR4 **(ensure that ethanol has been added before use).** Incubate at room temperature for 2 min, centrifuge at 12,000 rpm (~13,400 × g) for 30-60 sec, discard the waste liquid in the collection tube, and put Column CR4 back into the collection tube.
15. Repeat step14.
16. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min and discard the waste liquid. Place the Column CR4 at room temperature for several minutes to completely dry the residual washing buffer in the adsorption material.

    Note: After centrifugation, leave Column CR4 at room temperature for several minutes to fully dry the membrane. If there is Buffer RW residue, it may affect subsequent reverse transcription, fluorescence quantification and other experiments.
17. Transfer the adsorption column CR4 into a new RNase-Free centrifuge tube, add 30-50 µl RNase-Free ddH_2O and leave at room temperature for 2 min, centrifuge at 12,000 rpm (~13,400 × g) for 2 min to obtain the solution.

    Note: The volume of elution buffer should not be less than 30 µl. Too small a volume will affect the recovery of efficiency. RNA solution should be stored at -70°C.