RNAprep Pure Blood Kit

For purification of total RNA from human whole blood

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RNAprep Pure Blood Kit

(Spin Column)

Cat. no. 4992238

Kit Contents

Contents	4992238 50 preps
10 × Red Cell Lysis Buffer	60 ml
Buffer RL	30 ml
Buffer RW1	40 ml
Buffer RW	12 ml
RNase-Free DNase I (1500 U)	1
Buffer RDD (DNA Digest Buffer)	4 ml
RNase-Free ddH_2O	1 ml
RNase-Free ddH_2O	15 ml
RNase-Free Columns CR2 set	50
RNase-Free Columns CS set	50
RNase-Free Centrifuge Tubes (1.5 ml)	50
Handbook	1

Storage

RNase-Free DNase I, Buffer RDD and RNase-Free ddH_2O (Tubular) should be stored at 2-8°C for 15 months; Buffer RL/β-mercaptoethanol mix can be stored at 2-8°C for 1 month; others stored at room temperature (15-30°C) for 15 months.

Introduction

RNAprep pure Blood Kit provides a fast, simple, and cost-effective method for purification of total RNA from blood. The purified RNA is ready for use in downstream applications such as RT-PCR and real-time RT-PCR, micro array, northern blot, dot blot, polyA screening, in vitro transcription, and molecular cloning.

Notes of preventing RNase contamination

1. Change gloves regularly. Bacteria on the skin can result in RNase contamination.
2. Use RNase-Free plastic and tips to avoid cross-contamination.
3. RNA can be protected in Buffer RL, but RNA must be stored or applied in RNase-Free plastic or glassware. To wipe off RNase, the glassware can be roasted at 150°C for 4 hours, while plastic can be dipped in 0.5 M NaOH for 10 min, washed by water thoroughly, then sterilization.
4. Use RNase-Free water to confect solution.

Important points before starting

1. β-Mercaptoethanol (β-ME) must be added to Buffer RL before use. The final concentration of β-ME is 1%. For example, add 10 μl β-ME to 1 ml Buffer RL. Buffer RL containing β-ME can be stored at 2-8°C for up to 1 month. Buffer RL may form a precipitate upon storage. If necessary, re-dissolve by warming, and then place at room temperature (15-30°C).
2. Buffer RW is supplied as a concentrate. Before using for the first time, add ethanol (96-100%) as indicated on the bottle to obtain a working solution.
3. Blood and body fluids of all human subjects are considered potentially infectious. All necessary precautions should be taken when working with whole blood.
4. The maximum amount of human whole blood that can be processed (1.5 ml) has been determined for blood from healthy adults (approximately 4000-7000 leukocytes per microliter). Reduce amount appropriately if using blood with elevated numbers of leukocytes. A maximum of 1×10^7 leukocytes can be processed.

5. After leukocyte lysis, all steps of this protocol should be performed at room temperature (15-30°C), as quickly as possible.
6. Homogenized cell lysates (in Buffer RL) can be stored at -70°C. Frozen lysates should be incubated at 37°C in a water bath for 10 min until completely thawed and salts are dissolved. Continue with step 7.
7. Frozen whole blood cannot be used.

Preparation of DNase I stock solution

Dissolve the lyophilized DNase I (1500 U) in 550 µl of the RNase-Free ddH_2O (Tubular). Mix gently by inverting. Do not vortex. Divide it into single-use aliquots, and store at -30~-15°C for up to 9 months.

Note: Thawed aliquots can be stored at 2-8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Protocol

1. Red Cell Lysis Buffer is supplied as a concentrate. Please add appropriate volumes of Red Cell Lysis Buffer (200 µl volumes of blood sample should need 140 µl volumes of 10 × Red Cell Lysis Buffer). Dilute 10 × Red Cell Lysis Buffer to 1 × with RNase-Free ddH_2O to make a working solution.
2. Mix 1 volume of human whole blood with 5 volumes of 1 × Red Cell Lysis Buffer in an appropriately sized tube (not provided).

   Note: For optimal results, the volume of the mixture (blood + Buffer RL) should not exceed 3/4 of the volume of the tube to allow efficient mixing. Reduce amount appropriately if blood with elevated numbers of leukocytes is used, the amount of Buffer RL used in Step 7 should also be reduced.
3. Incubate for 10-15 min on ice. Mix by vortex briefly 2 times during incubation.

   Note: The cloudy suspension becomes translucent during incubation, indicating lysis of red cell. If necessary, incubation time can be extended to 20 min.
4. Centrifuge at 2,100 rpm (~400 × g) for 10 min at 4°C, and completely remove and discard supernatant.

   Note: Leukocytes will form a pellet after centrifugation. Ensure

supernatant is completely removed. Trace amounts of red cells, which give the pellet a red tint, will be eliminated in the following wash step.

5. Add 1 × Red Cell Lysis Buffer to the cell pellet (use 2 volumes of 1 × Red Cell Lysis Buffer per volume of whole blood used in step 1). Re-suspend cells by vortex briefly.
6. Centrifuge at 2100 rpm (~400 × g) for 10 min at 4°C, and completely remove and discard supernatant.

   Note: Incomplete removal of the supernatant will interfere with lysis and subsequent binding of RNA to the spin column, resulting in lower yield.
7. Add Buffer RL **(Ensure β-mercaptoethanol has been added to Buffer RL)** to pelleted leukocytes according to the table below. Vortex or pipet to mix.

   Note: When not using healthy blood, refer to number of leukocytes to determine the volume of Buffer RL required. Buffer RL disrupts the cells. No cell clumps should be visible before you proceed to the homogenization step.

Buffer RL (μl)	Healthy whole blood (ml)	Number of leukocytes
350	Up to 0.5	Up to 2×10^6
600	0.5 to 1.5	2×10^6 to 1×10^7

8. Transfer the entire lysate to an RNase-Free Filtration Column CS placed in a 2 ml Collection Tube (supplied). Close the lid gently, and centrifuge for 2 min at 12000 rpm (~13,400 × g). Discard the Filtration Column CS.

   Note: To avoid aerosol formation, adjust pipettor to 750 μl to ensure that the lysate can be added to the Filtration Column CS in a single step. If too many cells have been used, after homogenization the lysate will be too viscous to pipet.
9. Add 1 volume of 70% ethanol (usually 350 μl or 600 μl) to the cleared lysate, and mix immediately by pipetting. Transfer the sample, including any precipitate that may have formed, to an RNase-Free Spin Column CR2 placed in a 2 ml Collection Tube. Close the lid gently, and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through.

Note: please confect 70% ethanol with RNase-Free ddH_2O. If sample is lost partly, reduce accordingly the amount of 70% ethanol; if volume of the lysate is more than maximum amount of Spin Column CR2, divide the lysate into appropriate volume and flow several times.

10. Add 350 µl Buffer RW1 to the Spin Column CR2, close the lid gently, and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through.
11. Preparation of DNase I working solution: Add 10 µl DNase I stock solution (see Preparation of DNase I stock solution) to 70 µl Buffer RDD. Mix by gently inverting the tube.
12. Add the DNase I working solution (80 µl) directly to the center of Spin Column CR2, and place at room temperature (15-30°C) for 15 min.
13. Add 350 µl Buffer RW1 to the Spin Column CR2. Close the lid gently, and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through.
14. Add 500 µl Buffer RW to the Spin Column CR2 **(Ensure that ethanol is added to Buffer RW before use)**. Place at room temperature (15-25°C) for 2 min, and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through.
15. Repeat step 14.
16. Centrifuge for 2 min at 12,000 rpm (~13,400 × g) and incubate the Spin Column CR2 at room temperature (15-25°C) for several minutes to completely dry the spin column membrane.

    Note: The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.
17. Place the Spin Column CR2 in a new 1.5 ml Centrifuge Tube (supplied). Add 30-50 µl RNase-Free ddH_2O directly to the spin column membrane. Close the lid gently, place in room temperature (15-25°C) for 2 min and centrifuge for 2 min at 12,000 rpm (~13,400 × g) to elute the RNA.

    Note: Elution buffer should not be less than 30 µl, or else it will affect the elution efficiency. Purified RNA may be stored at -70°C.