EasyPrep Blood RNAprep Purification Kit

For purification of total RNA from human whole blood

Contents

Introduction

The EasyPrep Blood RNAprep Purification Kit provides a fast, simple, and cost-effective method for the purification of total RNA from blood. The purified RNA is ready for use in downstream applications such as reverse transcription polymerase chain reaction (RT-PCR) and real-time RT-PCR, microarray, Northern blotting, dot blot, polyA screening, in vitro transcription, and molecular cloning.

Notes for 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 RBL. However, RNA must be stored or RNA experiments must be conducted in RNase-free plastic or glassware. To remove/degrade RNase, glassware can be heated at 150℃ for 4 h, and plastic can be immersed in 0.5 M NaOH for 10 min, washed with water thoroughly, and then sterilized.
4. Use RNase-free water to confect solution.

Yield of total RNA purified using the EasyPrep Blood RNAprep Purification Kit

Sample	Volume	RNA Yields
Human whole blood	1 ml	3 μg
White blood cells	1 x 10^7	15–20 μg

Important points before starting the purification

1. β-Mercaptoethanol (β-ME) must be added to Buffer RBL before use. The final concentration of β-ME is 1%. For example, add 10 μL β-ME to 1 mL Buffer RBL. Buffer RBL containing β-ME can be stored at 4°C for up to 1 month. Buffer RBL may form a precipitate upon storage. If necessary, it can be redissolved by warming, and then, the buffer can be stored at room temperature (15°C–25°C).
2. Buffer RBW is supplied as a concentrate. Before use, add ethanol (96%–100%), as indicated on the bottle, to obtain a working solution.
3. Blood and body fluids from all human subjects are considered potentially infectious. All necessary precautions recommended by the Food and Drug Administration (in the United States), the Bundesseuchengesetz (in Germany), or the appropriate regulatory authorities in the country of use should be taken when working with whole blood.
4. The maximum volume 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 the volume appropriately if using blood with elevated leukocyte counts. A maximum of 1 × 10^7 leukocytes can be processed.
5. After erythrocyte lysis, all steps of this protocol should be performed at room temperature (15°C–

25°C), as quickly as possible.

6. Homogenized cell lysates from Step 6 can be stored at −70°C for several months. Frozen lysates should be incubated at 37°C in a water bath until completely thawed and salts are dissolved. Avoid prolonged incubation, which may compromise RNA integrity. Continue with Step 7.
7. Frozen whole blood cannot be used.

Preparation of DNase I stock solution

Dissolve the lyophilized DNase I (1500 Kunitz units) in 550 µL RNase-free ddH_2O (tubular). Mix gently by inverting. Do not vortex. Divide it into single-use aliquots, and store at −20°C for up to 9 months. Thawed aliquots can be stored at 2°C–8°C for up to 6 weeks.

Do not refreeze the aliquots after thawing.

Kit Contents

Contents	DPT-BD33 (50 preps)
10 x Red Cell Lysis Buffer	60 ml
Buffer RBL	30 mL
Buffer DBRW1	40 mL
Buffer RBW	12 mL
DNase I（1500 U）	1
Buffer RBDD	4 mL
RNase-free ddH_2O（Tubular）	1 mL
RNase-free ddH_2O（Bottled）	15 mL
RNase-free Spin Columns CR2	50
RNase-free Filtration Columns CS	50
RNase-free Collection Tubes （1.5 ml）	50

Storage

RNase-free DNase I, Buffer RBDD, and RNase-free ddH_2O (tubular) should be stored at 2°C–8°C; the Buffer RBL/β-mercaptoethanol mix can be stored at 4℃ for 1 month; and the remaining reagents should be stored at room temperature (15°C–25°C).

Protocol

1. Red Cell Lysis Buffer is supplied as a concentrate. Dilute 10× Red Cell lysis buffer to 1× with RNase-free ddH_2O to prepare a working solution.

Note: Every 200 µL of blood sample requires 1400 µL of 1× Red Cell Lysis Buffer for use totally. Scale up the 1× Red Cell Lysis Buffer according to sample volume.

2. Mix 1 volume of human whole blood with 5 volumes of 1× Red Cell Lysis Buffer in an appropriately sized tube (not supplied).

Note: For optimal results, the volume of the mixture (blood + Buffer RBL) should not exceed three-fourth of the volume of the tube to allow efficient mixing. Use an appropriate volume of whole blood. Up to 1.5 mL of healthy blood (typically 4000–7000 leukocytes per microliter) can be processed. Reduce the volume appropriately if blood with elevated numbers of leukocytes is used.

3. Incubate for 10–15 min on ice. Mix by vortexing briefly two times during incubation.

Note: The cloudy suspension becomes translucent during incubation, indicating the lysis of red blood cells. If necessary, incubation time can be extended to 20 min.

4. Centrifuge at 400 × g for 10 min at 4°C, and completely remove and discard the supernatant.

Note: Leukocytes will form a pellet after centrifugation. Ensure that the supernatant is completely removed. Trace amounts of red blood 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). Resuspend cells by vortexing briefly.
6. Centrifuge at 400 × g for 10 min at 4°C, and completely remove and discard the 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 RBL (ensure that β-ME is added to Buffer RBL) to pelleted leukocytes according to the table below. Vortex or pipet for mixing.

Note: When not using healthy blood, refer to the number of leukocytes to determine the volume of Buffer RBL required to be added. Buffer RBL disrupts the cells. No cell clumps should be visible before proceeding to the homogenization step. Vortex or pipet further to remove any clumps.

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

8. Transfer the entire lysate to an RNase-free CS spin column placed in a 2-mL collection tube (supplied). Close the lid gently, and centrifuge for 2 min at 12,000 rpm (~13,400 × g). Discard the CS spin column.

Note: To avoid aerosol formation, adjust the pipettor to 750 µL to ensure that the lysate can be added to the CS spin column 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 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 CR2 spin column 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 ddH2O. If the sample is lost partly, accordingly reduce the volume of 70% ethanol; if the volume of the lysate is more than the maximum volume that can be loaded onto the CR2 spin column, then divide the lysate in two.

10. Add 350 µL Buffer DBRW1 to the CR2 spin column, 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 RBDD. Mix by gently inverting the tube.
12. Add the DNase I working solution (80 µL) directly to the center of the CR2 spin column, and incubate it at room temperature (15°C–25°C) for 15 min.
13. Add 350 µL Buffer DBRW1 to the CR2 spin column. Close the lid gently, and centrifuge for 30–60 s at 12,000rpm (~13,400 × g). Discard the flow-through.
14. Add 500 µL Buffer RBW to the CR2 spin column (ensure that ethanol is added to Buffer RBW before use). Incubate at room temperature (15°C–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) to dry the spin column membrane.

Note: The long centrifugation protocol dries the spin column membrane, ensuring that all ethanol is removed during RNA elution. Residual ethanol may interfere with downstream reactions.

17. Place the CR2 spin column in a new 1.5-mL collection tube (supplied). Add 50–100 µL RNase-free water directly to the spin column membrane. Close the lid gently, incubate at room temperature (15°C–25°C) for 2 min, and centrifuge for 2 min at 12,000 rpm (~13,400 × g) to elute the RNA.

Note: If elution is performed in small volumes (<50 µL), the elution buffer must be dispensed to the center of the membrane for complete elution of bound RNA. Purified RNA may be stored at −70°C.