AXYGEN BIOSCIENCES

AxyPrep Blood Total RNA Miniprep Kit

For the purification of total RNA from blood

Kit contents, storage and stability

Cat. No.	AP-MN-BL-RNA-4	AP-MN-BL-RNA-50	AP-MN-BL-RNA-250
Kit size	4 preps	50 preps	250 preps
Spin/vac mini column	4	50	250
2 ml Microfuge tube	8	100	500
1.5 ml Microfuge tube	4	50	250
Buffer RL	14 ml	180 ml	2×450 ml
Buffer R-I	2 ml	24 ml	120 ml
Buffer R-II	1 ml	12 ml	60 ml
Buffer W1A concentrate	2.4 ml	24 ml	120 ml
Buffer W2 concentrate	2.4 ml	24 ml	2×72 ml
Buffer TE (DNase & RNase-free)	1 ml	6 ml	30 ml
Protocol manual	1	1	1

All buffers are stable for a period of at least 12 months from the date of receipt when stored under ambient conditions. Please avoid exposure to direct sunlight or extremes in temperature.

Buffer RL: Erythrocyte lysis buffer. Store at room temperature.

Buffer R-I: Cell lysis buffer. Store at room temperature.

Buffer R-II: Neutralization buffer. Store at room temperature.

Buffer W1A concentrate: Wash buffer. Before use of the kit, add the amount of ethanol specified on the bottle label. Either 100% or 95% denatured ethanol can be used. Mix well and store at room temperature.

Buffer W2 concentrate: Desalting buffer. Before use of the kit, add the amount of ethanol specified on the bottle label. Either 100% or 95% denatured ethanol can be used. Mix well and store at room temperature.

Buffer TE(DNase & RNase-free): Eluent. Contains 0.1 mM EDTA. Store at room temperature.

Axygen Biosciences 33210 Central Avenue, Union City, CA 94587 USA Tel:510-494-8900 Fax:510-494-0700 e-mail:support.axyprepkits@axygenbio.com web:www.axygenbio.com

Introduction

The AxyPrep Blood Total RNA Miniprep Kit represents a new approach to RNA purification. This kit is designed to eliminate the problems associated with other spin column-type RNA kits, such as clogged columns, incomplete purification, size-selection and degraded RNA. Following the lysis and removal of the erythrocytes, the leukocytes are lysed by Buffer R-I, which also nullifies any indigenous RNase activity. Cellular proteins and genomic DNA are then precipitated and removed by the addition of Neutralization Buffer R-II to the cell lysate. This eliminates most of the genomic DNA and cellular debris before the RNA is loaded onto the Spin/vac column (unlike other kits). The total cellular RNA is then bound to a Spin/vac miniprep column for further washing and desalting. Highly purified, full-length total cellular RNA representing the original mRNA pool is then eluted in a small volume of Buffer TE (DNase & RNase-free) (or DEPC-treated water) and is ready for use in any downstream application.

Caution

Buffer R-I, Buffer R-II and Buffer W1A contain chemical irritants. When working with these buffers, always wear protective clothing such as safety glasses, gloves and laboratory coat. Be careful to avoid contact with eyes and skin. In the case of such contact, wash immediately with water. If necessary, seek medical assistance.

Please follow established safety guidelines for handling blood samples and disposing of related waste.

Equipment and consumables required

- Microcentrifuge capable of 12,000×g
- AxyVac Vacuum Manifold (#AP-VM) or other vacuum manifold
- Vacuum regulator
- Vacuum source (capable of -25-30 inches Hg)
- 95-100% ethanol
- Isopropanol

Preparation before experiment

1) Before using the kit, add ethanol to both the Buffer W1A concentrate and the Buffer W2 concentrate as specified on the bottle labels. Mix well.
2) Use DEPC-treated materials whenever practical.

Protocols

I. Purification of Total RNA from whole blood

Buffer RL is used in a ratio of 1 ml per 200 μl of whole blood in the initial erythrocyte lysis step. This kit contains sufficient Buffer RL to process up to 400 μl of whole blood per prep. Processing 200 μl of whole blood will allow the contents to fit within the 2 ml microfuge tubes. To ensure optimal mixing and erythrocyte lysis, the total volume of [blood + Buffer RL] should not exceed 3/4 of the volume of the tube.

1. Mix 200-400 µl whole blood containing anticoagulant with 1-2 ml Buffer RL in an appropriately sized tube.

   Note: If volumes of blood between 200-400 µl are used, adjust the volume of Buffer RL accordingly.

2. Incubate for 10-15 minutes on ice. Mix by vortexing briefly, twice during incubation.
3. Centrifuge at 3,000×g for 5 minutes at 4°C. Using a pipetter, remove as much of the supernatant as possible without disturbing the pellet. Discard the supernatant.
4. Add 0.5 ml (200 µl initial blood volume) -to-1 ml (400 µl initial blood volume) Buffer RL to the leukocyte pellet. Resuspend the cells by vortexing briefly gently. Incubate on ice for 5 minutes.

   Note: If volumes of blood between 200-400 µl are used, adjust the volume of Buffer RL accordingly.

5. Centrifuge at 3,000×g for 5 minutes at 4°C. Completely remove and discard the supernatant.
6. Add 400 µl of Buffer R-I and mix thoroughly by pipetting up and down 5× or by vortexing for 15-30 seconds.
7. Add 200 µl of Buffer R-II and vortex for 1 minute. Centrifuge at 12,000×g for 10 minutes at 4°C.
8. Transfer the supernatant to a 2 ml microfuge tube (provided). Add 250 µl of isopropanol and mix well.

Proceed with either the A (centrifugation) protocol or B (vacuum) protocol, below.

A. Using centrifugation

9. Place a Spin/vac column into a 2 ml microfuge tube (provided). Transfer the binding mixture from Step 8 into the Spin/vac column. Centrifuge at 6,000×g for 1 minute at room temperature or 4°C.
10. Discard the filtrate from the 2 ml microfuge tube. Place the Spin/vac column back into the 2 ml microfuge tube. And add 700 µl of Buffer W1A to the Spin/vac column and centrifuge at 12,000×g for 1 minute.

    Note: Make sure that ethanol has been added into Buffer W1A concentrate. Make a notation on the bottle label for future reference.

11. Discard the filtrate and place the Spin/vac column back into the 2 ml microfuge tube. Add 700 µl of Buffer W2 and centrifuge at 12,000×g for 1 minute. Discard the filtrate from the 2 ml microfuge tube and repeat this wash with a second 700 µl aliquot of Buffer W2.

    Note: Make sure that ethanol has been added into Buffer W2 concentrate. Make a notation on the bottle label for future reference.

12. Discard filtrate from the 2 ml microfuge tube. Place the Spin/vac back into the 2 ml microfuge tube. Centrifuge at 12,000×g for 1 minute.
13. Transfer the Spin/vac column into a clean 1.5 ml microfuge tube (provided). Add 60-100 µl of Buffer TE (DNase & RNase-free) to the center of the membrane. Let it stand for 1 minute at room temperature. Centrifuge at 12,000×g for 1 minute.

B. Using vacuum

9. Attach the vacuum manifold base to a vacuum source. Firmly position the Spin/vac column(s) into the complimentary fittings on the manifold top. Transfer the binding mixture from Step 8 to the Spin/vac column. Turn on the vacuum source and adjust to -25 inches Hg. Continue to apply the vacuum until no solution remains in the Spin/vac column(s).
10. Add 700 μl of Buffer W1A and draw all of the solution through the Spin/vac column.

    Note: Make sure that ethanol has been added into Buffer W1A concentrate. Make a notation on the bottle label for future reference.

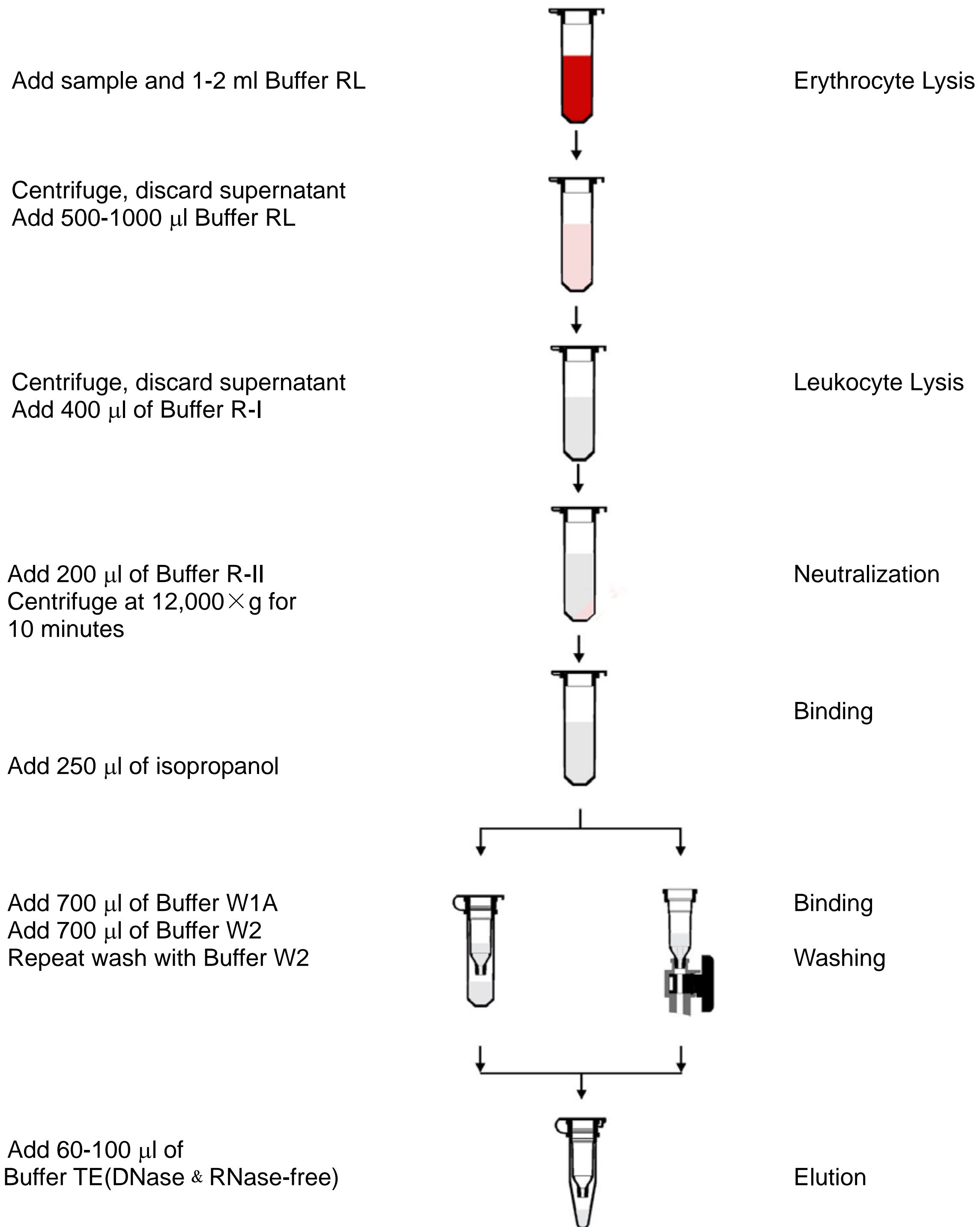
11. Add 700 μl of Buffer W2 along the wall of Spin/vac column to wash off residual Buffer W1A and draw all of the solution through the column. Repeat this wash with a second 700 μl aliquot of Buffer W2.

    Note: Make sure that ethanol has been added into Buffer W2 concentrate. Make a notation on the bottle label for future reference.

.

12. Transfer the Spin/vac column into a 2 ml microfuge tube（provided）and centrifuge at 12,000×g for 1 minute.
13. Transfer the Spin/vac column into a clean 1.5 ml microfuge tube (provided). Add 60-100 μl of Buffer TE (DNase & RNase-free) to the center of the membrane. Let it stand for 1 minute at room temperature. Centrifuge at 12,000×g for 1 minute.

Overview



Troubleshooting

1. Incomplete erythrocyte lysis

- The cloudy suspension does not become translucent in step 2: Extene incubation on ice to 20 minutes.
- Pellet in step 3 is red: Incubate for an additional 5-10 minutes on ice after addition of Buffer RL at step 4.

2. Little or no RNA eluted

- Old sample: use freshly collected blood sample.
- Too much starting material used: Repeat the elution with warmed Eluent.
- Incomplete removal of supernatant: Ensure complete removal of the supernatant after harvesting cells.

3. Low $A_{260/A280}$ value

- Inefficient cell lysis due to insufficient mixing of the samples with Buffer R-I.
- Buffer W1A or Buffer W2 prepared incorrectly: Add the correct amount of ethanol.

4. RNA degraded

- Blood sample stored for too long prior to RNA isolation.
- Inappropriate handling of starting material: Ensure that cells have been properly handled and the protocol has been performed without interruptions, especially the initial steps involving cell lysis.
- RNase contamination check for RNase contamination of buffers. Although all buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNase during the procedure or later handling.

Warranty/Disclaimer

Axygen Biosciences warrants that this kit will perform as indicated for the specified application for a period of up to 12 months from the date of receipt when stored in the specified manner indicated and used according to the instructions provided. In using this product, the customer agrees that Axygen Biosciences shall not be held liable for any direct or indirect damages, including, but not limited to, personal injury, property damage or lost profits (or other economic loss) resulting from the use or inability to use this product. In the event that this product fails to perform in the specified manner, remedial measures on the part of Axygen Biosciences shall be limited to the replacement of this product and will be implemented at the discretion of Axygen Biosciences.