PrimePrep™ Blood RNA Extraction Kit

Introduction

***PrimePrep*™ Blood RNA Extraction Kit** is provide a fast, easy method for the preparation of total cellular RNA from up to 1.5 ml of whole blood. Co ntaminants and enzyme inhibitors such as hemoglobin and heparin are co mpletely removed. *PrimePrep*™ Blood RNA Extraction Kit represent a tech nology for total RNA preparation that combines the selective binding prop erties of microfiber-silica-based membrane with centrifugation.
Purified total RNA ready for use in downstream applications such as RT-PCR, cDNA synthesis and real-time PCR etc.

Kit Components

Cat. No. / Reagents	KR-1000 (50 Prep.)
Spin column 1 **(Blue O-ring)**	50 ea
Spin column 2	50 ea
Collection tube	150 ea
Buffer BRR	200 ml x 2 bottles
Buffer BRL	40 ml
Buffer BRW1	20 ml
Buffer BRW2	11 ml
Buffer BRE	10 ml

Before you begin

- **Add 20 ml absolute ethanol to Buffer VRW1 before use.**
- **Add 44 ml absolute ethanol to Buffer VRW2 before use.**
- **Prepare β-mercaptoethanol (14.2 M), isopropanol, 1.5 ml microcentrifuge tube.**

! Caution:
- This product is for research use only.
- Buffer VRL and Buffer VRW1 contains strong denaturant. Be careful to avoid co ntacting with skin and eyes. In the case of such contact, wash immediately wi th plenty water.

GeNeT BIO GLOBAL GENE NETWORK
Tel. +82-0502-523-3484 **Fax.** +82-0502-523-3485
E-mail info@genetbio.co.kr **homepage** www.genetbio.com

Experimental Protocol

1. **Prepare 200 µl (1 volume) of whole blood with 1 ml (5 volumes) of Buffer BRR in a 1.5 ml microcentrifuge tube (appropriately sized).**
   · Add Buffer BRR to whole blood according to the table below.

Volume of whole blood	Volume of Buffer BRR	Centrifuge tube
200 µl	1.0 ml	1.5 ml
500 µl	2.5 ml	15 ml
1.0 ml	5.0 ml	
1.5 ml	7.5 ml	

2. **Incubate for 10~15 min on ice. Mix by vortexing briefly 2 times during incubation.**
   · The cloudy mixture becomes translucent during incubation, indicating lysis of erythrocytes.
3. **Centrifuge at 3,000 rpm for 10 min at 4 ℃, remove supernatant using micropipette.**

   • Leukocytes will form a pellet after centrifugation. Some amounts of ery throcytes, which give the pellet a red tint, will be completely remove in the ste p 5.
4. **Add 400 µl of Buffer BRR (2 volumes of whole blood used in step 1) to the cell pellet. Resuspend cells by pulse vortexing.**
5. **Centrifuge at 3,000 rpm for 10 min at 4 ℃, completely remove supernatant using micropipette.**
6. **Add 350 µl of Buffer BRL and 3.5 µl of β-mercaptoethanol (β-ME, 14.2 M) to pelleted leukocytes according to the table below. Mix by vortexing for 10~15 sec. After this step, briefly centrifuge the tube to remove drops from the inside of the lid.**

Volume of whole blood	Volume of Buffer BRL	Volume of β-ME
~ 0.5 ml	350 µl	3.5 µl
0.5 ~ 1.5 ml	600 µl	6.0 µl

   · β-ME must be add to Buffer BRL as ratio of 10 ㎕ of β-ME per 1 ml of Buffer BRL.
   · Check Buffer BRL for precipitation. If happened, dissolve precipitate completely by incubation at 40~50 °C until dissolve.

7. Transfer the lysate into Spin Column 1 (Blue O-ring), and centrifuge for 2 min at maximum rpm in table centrifuge. After centrifugation, transfer th e flow-through into new 1.5 ml microcentrifuge tube (not provided).

· If too many cells have been used, mixture remain in spin column 1. Additionally centrifugation for 2 min at maximum rpm.

8. Add 1 volume (350 or 600 µl) of 70% ethanol to the homogenized lysate. Mix by vortexing for 5 sec.

9. Centrifuge at 3,000 rpm for 5 sec at room temperature.

· For protect from contamination by carryover, spin-down the tube to remove dr ops from the inside of the lid.

10. Transfer the mixture to Spin Column 2 (fit in a 2 ml collection tube).

· Maximum loading volume is 700 µl. If used 600 µl of Buffer BRL, transfer the m ixture to Spin Column 2 for two times. Repeat step 10 and 11.

11. Centrifuge at 13,000 rpm for 30 sec at room temperature. And discard the pass-through and reinsert the Spin column 2 into the same collection tube.

12.Add 700 µl of Buffer BRW1 to the Spin column 2. Centrifuge at 13,000 rp m for 30 sec at room temperature. And discard the pass-through and reins ert the Spin column 2 into the new collection tube.

13.Add 500 µl of Buffer BRW2 to the Spin column 2. Centrifuge at 13,000 rp m for 30 sec at room temperature. And discard the pass-through and reins ert the Spin column 2 into the new collection tube.

14. Repeat step 13.

15.Centrifuge at 13,000 rpm for 1 min at room temperature to remove resi dual wash buffer. Transfer the Spin column 2 to a new collection tube.

· Residual ethanol may interfere with downstream reactions.

16.Add 30~50 µl of Buffer BRE to the center of the membrane in Spin Colu mn 2, and wait for at least 1 min at room temperature.

17. Centrifuge at 13,000 rpm for 1 min at room temperature.

·Purified RNA can be stored at -20 ℃ for immediate use and stored at -70 ℃ f or long term storage.

*** Microfiber-silica-membrane technology of *PrimePrep*™ Blood RNA Extraction Kit is eff iciently removes most of the DNA. However, spin column system might be elute with small amount genomic DNA. Recommend DNase treatment is efficient for more comple tely DNA removal may be necessary for RNA applications that are sensitive to very sma ll amounts of DNA. Generally, DNase I (RNase-free) treatment is used for remove DNA. But, purified RNA yield also reduced.**