TIANGEN®

RNALock Reagent

For immediate stabilization of the gene expression profile in the whole blood

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RNALock Reagent

Cat. no. 4992731

Kit Contents

Contents	4992731
RNALock Reagent	100 ml
Buffer RSB	25 ml
Proteinase K	2 ml
Handbook	1

Storage Conditions

RNALock Reagent should be stored at room temperature (15-25°C).

Introduction

RNALock Reagent is liquid and nontoxic reagent for immediate preservation of RNA in fresh whole blood, enabling reliable gene expression analysis. The reagent preserves RNA from healthy human whole blood for up to 5 days at 2-8°C or at least 3 months at -20°C or -70°C. Mammalian whole blood with the reagent could be stored for 2 days at 15-25°C, 7 days at 2-8°C, or at least 6 months at -20°C or -70°C. During the storage, RNA remains intact and undegraded.

RNAprep Pure Hi-Blood Kit (Not supplied, Cat.no. 4992903) could be used for downstream purification of RNA from the blood stored in RNALock reagent with the optimal protocol indicated in RNALock reagent handbook. The purified RNA has high quality and no contamination with proteins and other purities and suitable for many downstream experiments such as RT-PCR, real time RT-PCR, chip analysis, Northern Blot, Dot blot, polyA screening, *in vitro* translation, RPA, cloning, etc.

RNA Yield for reference

Combined with RNAprep Pure Hi-Blood Kit and optimal protocol in RNALock Reagent handbook, RNA yield is as below:

Whole blood	Volume (μl)	Yield (μg)
Human	300	0.5-2*
Rat	100	2-6
Mouse	100	4-8

*RNA yield depends on individual donor for human whole blood.

Notes of preventing RNase contamination

1. Wear gloves when handling RNA and all reagents, as skin is a common source of RNase. Change gloves frequently. Use sterilized plastic ware and tips to avoid cross-contamination.
2. Plastic or glass ware should be RNase-free. To wipe off RNase, the glassware could be dried at 150°C for 4 hours, while plastics could be dipped in 0.5 M NaOH for 10 min, and washed by RNA-Free ddH_2O thoroughly and sterilized.
3. Prepare the reagents with DEPC treated RNase-Free ddH_2O. Put ddH_2O in clean glassware, add 0.1% (v/v) DEPC and stand for one night after mixing thoroughly. Autoclave to remove any trace of DEPC.

Protocol

I. Preservation of the whole blood

a. Ensure RNALock reagent is stored at room temperature before use. Add 3 volumes of RNALock reagent to one volume of fresh anticoagulant-treated whole blood. (For example, add 900 µl RNALock reagent to 300 µl fresh human whole blood or 300 µl RNALock reagent to 100 µl fresh mammalian whole bloods).

Note: for preservation of the whole blood, add 3 volumes of RNALock reagent to fresh anticoagulant-treated blood immediately. Appropriate consumables could be used for large volume blood.

b. Close the lid immediately and invert for 8-10 times up and down. The reagent preserves RNA from healthy human whole blood for up to 5 days at 2-8°C or at least 3 months at -20°C. Mammalian whole blood with the reagent could be stored for 2 days at 15-25°C, 7 days at 2-8°C, or at least 6 months at -20°C.

Note: Blood sample with RNALock reagent has to be incubated for 2 hours at room temperature to make blood sample fully lytic. This should be performed before storage at low temperature or after the addition of RNALock reagent.

II. RNA Purification

Note: RNAprep Pure Hi-Blood Kit (Not supplied, Cat.no. 4992903) could be used for purification of RNA from the blood stored in RNALock reagent with the optimal protocol indicated in RNALock reagent handbook.

1. Keep the blood sample with RNALock reagent at room temperature or heat the sample up to room temperature in the 37°C water bath when purifying RNA from blood samples stored in the RNALock reagent. Centrifuge at 6,600 rpm (~4000 × g) for 10 min, and completely remove and discard supernatant.
2. Add 1 ml RNase-Free ddH_2O into the pellet, and pipet repeatedly to dissolve any clumps.
3. Centrifuge at 6,600 rpm (~4000 × g) for 10 min, and completely discard supernatant.

Note: Incomplete removal of the supernatant will interfere with subsequent binding of RNA to the CR4 spin column, resulting in lower yield.

4. Add 240 µl of Buffer RSB slowly and pipet repeatedly to dissolve any clumps.

   Note: The pellet is difficult to dissolve and incomplete lysis will result in lower yield.

5. Add 200 µl Buffer RHL (**please add β-mercaptoethanol before use**), 20 µl Proteinase K and mix thoroughly. Incubate for 10 min at 55°C. Invert up and down several times during incubation. No clumps should be visible.

   Note: Buffer RLH and Proteinase K should not be mixed in advance. Incubate for longer time in order to remove any clumps if the lysate is not homogenous.

6. Transfer the entire lysate to an RNase-Free spin column CS 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 spin column CS. Transfer the filtrate in 2 ml Collection Tube to a new RNase-Free centrifuge tube and avoid pipetting any cell debris.

7. Add 0.5 × volume ethanol (96%-100%) (usually 220 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 CR4 placed in a 2 ml Collection Tube. Close the lid gently, and centrifuge for 1 min at 12,000 rpm (~13,400 × g). Discard the flow-through.

8. Add 350 µl Buffer RW1H to the Spin Column CR4, Close the lid gently, and centrifuge for 30-60 sec at 12,000 rpm (~13,400 × g). Discard the flow-through.

9. Preparation of DNase I working solution: Add 10 µl DNase I stock solution to 70 µl Buffer RDD. Mix by gently inverting the tube. (Dissolve the lyophilized DNase I (1500 Kunitz units) 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 -20°C for up to 9 months.)

   Note: Thawed aliquots can be stored at 4 °C for up to 6 weeks. Do not refreeze the aliquots after thawing.

10. Add the DNase I working solution (80 µl) directly to the center of Spin Column CR4, and place at room temperature (15-25°C) for 15 min.

11. Add 350 µl Buffer RW1H to the Spin Column CR4. Close the lid gently, and centrifuge for 30-60 sec at 12,000 rpm (~13,400 × g). Discard the flow-through.
12. Add 500 µl Buffer RW **(Ensure that ethanol has been added to Buffer RW before use)** to the Spin Column CR4. Place at room temperature (15-25 °C) for 2 min, and centrifuge for 2 min at 12,000 rpm (~13,400 × g). Discard the flow-through.
13. Repeat step 12.
14. Centrifuge for 2 min at 12,000 rpm (~13,400 × g) and place at room temperature for 3 min to dry the spin column membrane thoroughly.

    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 Enzyme-catalyzed reactions.
15. Place the Spin Column CR4 in a new 1.5 ml Collection Tube (supplied). Add 30-50 µl RNase-Free ddH_2O directly to the spin column membrane. Close the lid gently, place at 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:RNase-Free ddH_2O should not be less than 30 µl. Lower volume will result in low yield. Purified RNA may be stored at -70°C.

III. Genomic DNA purification in blood sample

For the purification of genomic DNA in blood sample with RNALock reagent, please ask for protocol from TIANGEN BIOTECH (BEIJING) CO, LTD.