AFTSpin Blood Fast RNA Extraction Kit

Cat. No. : RK30125

Product components

Components	Component number	Size 50 RXN	Storage
Buffer RL3	RM30210	30 mL	RT
10X Red Blood Cell Lysis Buffer	RM30203	25 mL	RT
Buffer PR2	RM30141	35 mL	RT
RNase-free H_2O	RM30142	5 mL	RT
Buffer WB2	RM30144	12 mL	RT
RNase-free Adsorption Column and Collection Tubes	RM30185	50 pcs	RT
1.5 mL RNase-free centrifuge Tubes	RM30202	50 pcs	RT

Product Description

Red blood cell lysis buffer selectively lyses red blood cells, and then the unique lysis buffer / β-mercaptoethanol rapidly lyses white blood cells and inactivates cell RNA enzymes. After adjusting the binding conditions with ethanol, the RNA is selectively bound to the silicon membrane of the adsorption column in the state of high disordered salt, and then the cell metabolites, proteins and other impurities are removed by a series of rapid washing-centrifugation steps. Finally the low-salt RNase-free H_2O elutes the pure RNA from the silicon membrane.

Storage

This kit is guaranteed stable for 12 months when stored properly at room temperature. Low temperature storage is easy to cause sediment formation and affect the experimental results. Transportation at room temperature.

Precautions

1. This product is for scientific research use only by professionals and is not intended for clinical diagnosis or treatment.
2. Please wear a lab coat and disposable gloves for your safety and health.
3. Unless otherwise specified, all centrifugation steps should be completed at room temperature, using a benchtop centrifuge at a speed greater than or equal to 13,000 rpm (~14,000 x g).
4. Buffer RL3 and Buffer PR2 contain irritating compounds. Please wear latex gloves during operation to avoid contamination of skin, eyes and clothing. If skin, eyes are contaminated, rinse with plenty of water or normal saline.
5. After each use, the reagent bottle should be tightly capped immediately to avoid volatilization, oxidation and pH change caused by long-term exposure to the air.
6. The kit removes the vast majority of DNA contamination in the system, and purified RNA can usually be used for downstream experimental operations without DNase I treatment. If downstream experiments are sensitive to trace DNA, DNase I can be used to further remove DNA contamination.
7. Please wear a lab coat, disposable latex gloves, disposable mask, and use RNase-free consumables to avoid RNase contamination.

Operation Instruction

Preparation before the experiment

1. Prior to the first use, add 48 mL of absolute ethanol (self-prepared by the user) to Buffer WB2 and mix thoroughly. Mark the reagent bottle to indicate that ethanol has already been added.
2. Check Buffer RL3 for precipitate. If there is precipitate, put the tube in a water bath at 65℃ until the precipitate disappears.

User Protocol (please read the precautions first)

1. Add 1 volume (<1.5 ml) of various anticoagulant fresh blood (after mixing upside down) and 3 volume of red blood cell lysis buffer into an RNase-free centrifuge tube of appropriate size, and mix it upside down and gently flip the tube wall to ensure the mixing.

   Note: Before use, dilute 10X Red Blood Cell Lysis Buffer to 1X with DEPC treated water or RNase-free H_2O. Add β-mercaptoethanol to Buffer RL3 to the final concentration of 1%, such as adding 10 μl β-mercaptoethanol to 1 ml Buffer RL3. Try to prepare as needed.

2. Incubate at room temperature for 10 minutes (Flip the mixture several times to help break the red blood cells).
3. Centrifuge at 13,000 rpm (~14,000 x g) for 20 sec, discard the red supernatant, and carefully remove as much of the supernatant as possible (be careful not to remove the cell mass), leaving a complete white blood cell mass at the bottom of the tube.

   Note: After centrifugation, white leukocyte masses should be seen at the bottom of the tube, and there may be some red cell fragments together with the leukocyte masses. However, if most red cell masses appears, it indicates that the red blood cell lysis is not sufficient, and the cell masses should be re-suspended with the red blood cell lysis buffer and step 2 and 3 should be repeated. The supernatant should be removed and discarded as much as possible. Excessive residue will dilute the lysis buffer, resulting in decreased yield and purity.

4. Vortex or flick the tube wall to completely loosen and resuspend the white blood cells. Add 350 μL (< 0.5 mL whole blood) or 600 μL (0.5-1.5 mL whole blood) Buffer RL3, blow and mix well, and violently shake 20 sec by hand to fully lysis.

   Note: The number of white blood cells in the normal blood sample of the patient is 4000-7000 / μL, if the number of white blood cells in the blood sample may increase significantly, the amount of samples should be appropriately reduced. Alternatively, add Buffer RL3 in the ratio of 350 μL (<$2x10^6$ leucocytes) or 600 μL ($2x10^6$-$1x10^7$ leucocytes).

5. Blow and mix with pipette tip to help lysis or violently vortex until satisfactory homogenization are obtained (or electric homogenization for 30 sec). This can cut DNA, reduce viscosity and increase yield.
6. Add 0.5 times the volume of absolute ethanol to the lysis mixture, (precipitation may occur at this time, but it does not affect the extraction process), mix immediately and do not centrifuge.
7. Add the mixture to the RNase-free Adsorption Column placed in the Collection Tube, centrifuge at 13,000 rpm (14,000 x g) for 30 sec, and pour out the filtrate.

   Note:The adsorption column volume is 700 μL, and samples with a volume greater than 700 μL can be added in batches.

8. Place back the RNase-free Adsorption Column into the collection tube, add 700 μL of **Buffer PR2** to the Adsorption Column. Then centrifuge at 13,000 rpm (14,000 x g) for 30 sec, and discard the filtrate.
9. Place back the RNase-free Adsorption Column into the collection tube, add 500 μL of **Buffer WB2** (**confirm adding 48 mL of absolute ethanol before first use**) to the Adsorption Column, centrifuge at 13,000 rpm (14,000 x g) for 30 sec, and discard the filtrate.
10. Repeat Step 9 once.
11. Place back the RNase-free Adsorption Column into the collection tube, centrifuge the empty tube at 13,000 rpm (~14,000 x g) for 2 min to remove the remaining **Bufferd WB2** in the Adsorption Column.
12. Remove the RNase-free Adsorption Column and put it into a 1.5mL RNase-free centrifuge tube. Add 30-50 μL of RNase-free H_2O to the middle of the adsorption column and allow to stand at room temperature for 2 min. Centrifuge at 13,000 rpm (14,000 x g) for 1 min to elute the RNA.

    Note: The volume of elute buffer should not be less than 30 μL, otherwise it will affect the recovery efficiency. In order to increase the obtained RNA, add the centrifuged RNA back to the Adsorption Column for one more elution..

13. The extracted RNA can be directly used for downstream experiments or stored at -80℃.