AFTSpin Bacterial Fast RNA Extraction Kit

Cat. No. : RK30123

ABclonal Technology

Product components

Components	Component number	Size 50 RXN	Storage
Buffer RL1	RM30140	25 mL	RT
Buffer PR2	RM30141	35 mL	RT
lysozyme	RM30205	20 mg	4℃
Buffer WB2	RM30144	12 mL	RT
RNase-free Adsorption Column and Collection Tubes	RM30185	50 pcs	RT
gDNA Remove Column and Collection Tubes	RM30186	50 pcs	RT
1.5 mL RNase-free Centrifuge Tubes	RM30202	50 pcs	RT
TE (pH 8.0)	RM30204	6 mL	RT
RNase-free H_2O	RM30142	5 mL	RT

Product Description

This kit can be used for rapid extraction of RNA from various bacterial samples, and does not rely on toxic reagents such as phenol and chloroform. The unique lysis buffer rapidly lyses cells from trace samples and inactivates cell RNA enzymes. The lysate mixture passes through a gDNA Remove Column, where gDNA is removed and RNA is filtered through. After adjusting the binding conditions by ethanol, RNA is selectively bound to the silicon membrane of the adsorption column in the state of high disordered salt. In a series of rapid washing-centrifugation steps, the purified RNA is eluted from the silicon membrane with low salt RNase-free H_2O. The extracted RNA can be directly used in RT-PCR, qPCR and RNA library constructing experiments.

Storage

This kit is guaranteed stable for 12 months when stored properly at room temperature(15℃~25℃). Low temperature storage is easy to cause sediment formation and affect the experimental results. When sediment occurs, place the reagent tube(s) into the water bath (37℃) till solution becomes clear.

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 RL1 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 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 RL1 for precipitate. If there is precipitate, put the tube in a water bath at 65℃ until the precipitate disappears.
3. Before the experiment, add lysozyme or lysostaphin to TE (10 mM Tris-HCl， 1 mM EDTA) to reach a final concentration of 1 mg/mL for lysozyme or lysostaphin (For example, add 1mg lysozyme to 1mL TE). Try to prepare fresh enzyme mix in order to avoid inactivation. If the lysozyme solution mix is not used up, store it at -20℃.

User Protocol (please read the precautions first)

1. Centrifuge at 13,000 rpm (~14,000 x g) for 30 sec. Collect 1-2 mL of bacterial solution (10^8-10^9 cells) into a 1.5 mL centrifuge tube by centrifugation for 30 sec at 13,000 rpm (~14,000 x g). Remove as much supernatant as possible (incomplete removal of supernatant may affect the effect of downstream lysis of cell wall). According to the type and number of cells, take 100~200* μL of TE** to fully resuspend cells.

   *Note: Add 100 μL TE to every 5x10^8 cells, and add 200 μL TE to 5x10^8-7.5x10^8 cells.

   **Note: Lysozyme or lysostaphin has been added to TE at a concentration of 1 mg/mL.
2. Incubate lysozyme at room temperature (15-25℃) for 5 min or incubate lysostaphin (self-prepared) at 37℃ for 15 min to break cell wall. Vortex for 10 sec every 2 min to help break cell wall.

   Note: The difficulty of bacterial wall breaking varies. Generally, the above conditions are sufficient for the use of Gram negative bacteria *E. coli*, and this step may even be omitted. However, some Gram positive bacteria such as *B. subtilis* need to increase the concentration of lysozyme to 15 mg/mL and incubate for 10 minutes to break the wall. If it is *Staphylococcus aureus*, lysostaphin needs to be added to 1 mg/mL and incubated at 37 ℃ for 15 minutes.
3. Transient centrifugation. Colletct the cells to the bottom of the tube, remove and discard the supernatant, and vortex to resuspend the cells.
4. Add 500μL **Buffer RL1**, mix well, and vigorously shake 20 sec by hand to fully lysis.
5. Transfer the lysis mixture to the gDNA Remove Column placed in the Collection Tube, centrifuge at 13,000 rpm (~14,000 x g) for 30 sec, discard the gDNA Remove Column, and collect the filtrate (RNA in the filtrate).
6. Add 0.5 times the volume of absolute ethanol to the filtrate (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 discard the filtrate.
8. Place back the RNase-free Adsorption Column into the Collection Tube, add 700 μL of **Buffer PR2** to the Adsorption Column, incubate it for 30 sec at room temperature. 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 already adding 48 mL of absolute ethanol prior to the 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 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℃.