AFTSpin Tissue/Cell Fast miRNA Extraction Kit

Cat. No. : RK30126

ABclonal Technology

Product components

Components	Component number	Size 50 RXN	Storage
Buffer LB	RM30279	50 mL	4℃, protected from light
Buffer PR3*	RM30303	12 mL	RT
Buffer WB3**	RM30280	10 mL	RT
RNA Adsorption Column and Collection Tubes***	RM30281	50 pk	RT
miRNA Adsorption Column and Collection Tubes***	RM30282	50 pk	RT
RNase-free H_2O	RM30142	10 mL	RT

***Add 28 mL of absolute ethanol to 50 RXN Buffer PR3 before use.**

****Add 42 mL of absolute ethanol to 50 RXN Buffer WB3 before use.**

*****This kit provides RNA Adsorption Columns and miRNA Adsorption Columns, which are suitable for total RNA extraction and microRNA enrichment respectively.**

Product Description

This kit is suitable for the rapid extraction of microRNA and other small RNAs from various cells and tissues. It uses a unique lysis solution to rapidly lyse cells and inactivate cellular RNases. Organic extraction is employed to remove proteins and DNA. The special silicon - based matrix membrane in the spin column can adsorb total RNA, microRNA, and other various small RNAs. Subsequently, through a series of rapid washing-centrifugation steps, cell metabolites, proteins, and other impurities are further removed. Finally, a low - salt elution buffer is used to elute pure RNA from the silicon - based matrix membrane.

Storage

Buffer LB: Store at 4°C and protect from light;

Other reagents: Store at room temperature.

Precautions

1. Precipitation may appear in Buffer WB3 after adding ethanol and using it for a few days. This does not affect its use. You can directly pipette the supernatant for use.
2. After using each solution, close the lid promptly to avoid volatilization, oxidation, and changes in pH value caused by long-term exposure to air.
3. All centrifugation steps should be completed at room temperature, using a centrifuge with a maximum speed of 13,000 rpm.
4. Buffer LB and Buffer PR3 contain irritating compounds. When operating, wear latex gloves to avoid contact with skin, eyes, and clothing. In case of contact with skin or eyes, rinse immediately with a large amount of clean water or normal saline.
5. Different methods can be selected for different experiments. For example, for Northern Blot or expression microarray profiling, total RNA including microRNA can be extracted. The microRNA extracted by the enrichment method has removed larger fragments of mRNA and rRNA, etc., which can reduce the amplification background of certain downstream experiments. Only when the background is high or there is a large amount of non- specific amplification, try to use the microRNA extracted by the enrichment method.

Operation Description

Experimental Preparation

1. Self - prepared materials: absolute ethanol, chloroform.
2. Before the first use, add the specified amount of ethanol to the Buffer PR3 bottle and Buffer WB3 bottle. Mark them promptly

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after adding to avoid adding ethanol repeatedly!

Sample Processing

1. **Suspension cells**: Collect the cells, completely aspirate the culture medium. Flick the tube wall gently to completely loosen and resuspend the cell pellet. Add 1 mL of Buffer LB to every 5×10^6-1×10^7 cells, then vortex or pipette to fully mix and lyse them.

   Adherent cells: Aspirate all the remaining culture medium as thoroughly as possible. Add 1 mL of Buffer LB to each 10 cm^2 culture dish. Quickly shake gently to ensure that Buffer LB fully contacts all the cells at the bottom of the dish to lyse the cells and inactivate RNase. Gently pipette up and down repeatedly to mix well.

   Note: Adherent cells often do not completely detach from the culture flask (dish), but this does not mean incomplete lysis. At this time, the cell membrane has actually completely ruptured and released RNA. You can continue with the subsequent experiments.

   Animal and plant tissues: Use a scalpel to quickly cut the fresh tissue into small pieces. According to the mass of the tissue to be processed, add 1 mL of Buffer LB to every 50-100 mg of tissue, and then homogenize it thoroughly by electric or manual means. Alternatively, grind the tissue into a fine powder in liquid nitrogen, then transfer an appropriate amount of tissue fine powder (about 50-100 mg) into a 1.5 mL centrifuge tube containing 1 mL of Buffer LB, and vigorously pipette and vortex to mix it into a homogenate.
2. Incubate at room temperature for 5 minutes to fully separate the nucleic acid - protein complex.
3. Add 200 μL of chloroform and shake vigorously for 15 seconds.
4. Incubate at room temperature for 2-3 minutes, then centrifuge at 13,000 rpm for 10 minutes.
5. Carefully transfer the supernatant (approximately 600 μL) into a new centrifuge tube.

Total RNA Extraction (Extracting Total RNA Containing microRNA)

1. Add 1.5 times the volume of absolute ethanol stored at room temperature (usually 900 μL) to the supernatant, and vortex to mix well.

   Note: Precipitation may occur at this time, but it does not affect the extraction process. Immediately pipette to mix well.
2. Add the mixture (less than 700 μL each time, can be added in batches) to an **RNA adsorption column** (place the adsorption column in a collection tube), centrifuge at 12,000 rpm for 30 - 60 seconds, and discard the waste liquid.
3. Add 700 μL of Buffer PR3 (**please check if absolute ethanol has been added first!**), centrifuge at 12,000 rpm for 30 seconds, and **discard the waste liquid**.
4. Add 500 μL of Buffer WB3 (**please check if absolute ethanol has been added first!**), centrifuge at 12,000 rpm for 30 seconds, and **discard the waste liquid**.
5. Repeat step 4.
6. Place the **RNA adsorption column** back into the empty collection tube, centrifuge at 13,000 rpm for 2 minutes to remove the washing buffer as much as possible, to avoid the residual ethanol in the washing buffer inhibiting downstream reactions.
7. Take out the **RNA adsorption column** and place it in an RNase-free centrifuge tube. Add 30 - 50 μL of RNase-free H_2O to **the middle part of the adsorption membrane**, let it stand at room temperature for 1 minute, and then centrifuge at 13,000 rpm for 1 minute.

   Note: The recovery yield can be increased in the following ways: (1) Preheat RNase - free H_2O at 100℃; (2) Load the RNA filtrate onto the column again, let it stand at room temperature for 2 minutes, and then elute.
8. The RNA solution can be stored at -80℃ for long-term storage.

microRNA Enrichment (Extract only microRNA, not including other RNAs > 200 nt)

1. Add 0.5 times the volume of absolute ethanol **stored at room temperature** to the supernatant, and mix thoroughly by vortexing or pipetting.

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2. Add the mixture (less than 700 μL each time, can be added in batches) to an **RNA adsorption column** (place the adsorption column in a collection tube), centrifuge at 13,000 rpm for 30 - 60 seconds, and **collect the filtrate**.

   Note: The filtrate contains microRNA. The total RNA on the adsorption column, excluding microRNA, can be processed according to steps 3 - 7 of the "Total RNA Extraction" procedure to elute and recover the total RNA without microRNA.

3. Combine the filtrates, estimate the volume of the filtrate, and add 0.65 times the volume of absolute ethanol **stored at room temperature**. Mix thoroughly by vortexing or pipetting.
4. Add the mixture from the previous step (less than 700 μL each time, can be added in batches) to a **microRNA adsorption column** (place the adsorption column in a collection tube), centrifuge at 13,000 rpm for 30 seconds, and **discard the waste liquid**.
5. Add 700 μL of Buffer PR3 (**please check if absolute ethanol has been added first!**), centrifuge at 12,000 rpm for 30 seconds, and **discard the waste liquid**.
6. Add 500 μL of Buffer WB3 (**please check if absolute ethanol has been added first!**), centrifuge at 12,000 rpm for 30 seconds, and **discard the waste liquid**.
7. Repeat step 6.
8. Place the **microRNA adsorption column** back into the empty collection tube, centrifuge at 13,000 rpm for 2 minutes to remove the washing sbuffer as much as possible, to avoid the residual ethanol in the washing buffer inhibiting downstream reactions.
9. Take out the **microRNA adsorption column** and place it in an RNase-free centrifuge tube. Add 30-50 μL of RNase-free H_2O to **the middle part of the adsorption membrane**, let it stand at room temperature for 1 minute, and then centrifuge at 13,000 rpm for 1 minute.

   Note: The recovery yield can be increased in the following ways: (1) Preheat RNase-free H_2O at 100℃; (2) Load the RNA filtrate onto the column again, let it stand at room temperature for 2 minutes, and then elute.

10. The RNA solution can be stored at -80℃ for long-term storage.

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