# Column extraction of tissue cell miRNA kit

**Cat:** R2220
**Package:** 25T/50T/100T
**Storage:** RT, Valid for 1 year.

**Product composition:**

| Kit composition | 25T | 50T | 100T |
|---|---|---|---|
| Lysate | 16mL | 27mL | 55mL |
| Buffer B | 6.5mL | 13mL | 26mL |
| Bleach solution | 5mL | 10mL | 20mL |
| Euate | 1mL | 2mL | 4mL |
| miRNA adsorption column | 50 | 100 | 200 |
| 2mL collection tube | 50 | 100 | 200 |
| Specification | 1 | 1 | 1 |

**Notes:**

1. Before use, please add isopropyl alcohol to the rinse solution. Please refer to the label on the bottle.
2. Prepare anhydrous ethanol, isopropyl alcohol and 1×PBS.

**Product description:**

The tissue cell miRNA extraction kit produced by Solarbio is a new generation of products developed specifically for miRNA extraction, with the advantage that it is non-toxic and harmless. In addition, more than 95% of the Small RNA extracted by the kit is in the range of 15 ~ 200nt, and basically does not contain large RNA and DNA. The lytic solution in this kit has been developed and improved for a long time, with stronger lytic ability and higher extraction sensitivity. The adsorption column in the kit uses a special silicon matrix membrane filler, which greatly enhances its adsorption capacity for RNA, especially small RNA (<200nt). The resulting RNA is more pure and of higher quality. The extraction is simple and fast, the whole process can be completed in about 30min, the purity is good, the yield is high, and it can be directly used in various downstream experiments.

**Product features:**

1. Completely non-toxic, no need for phenol chloroform and other organic reagents extraction.
2. Wide application range, can be used for the extraction and purification of miRNA and total RNA of a variety of samples.
3. The operation is simple and fast, and the whole process is about 30 minutes.
4. With good purity and high yield, it can be directly used in various downstream experiments.

**Operation procedure:**

**一、Animal tissue sample extraction**

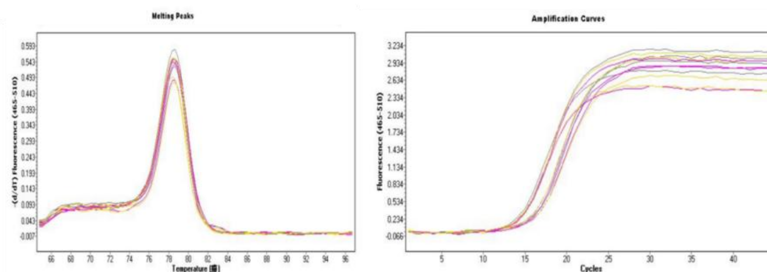
1. Fully grind and crush the tissue under the condition of liquid nitrogen, weigh a certain amount of sample (10mg~35mg) in 1.5mL EP tube, add 500μL lysate, and mix until the tissue powder is completely dissolved in the lysate. Let stand at room temperature for 5min to fully lysate cells.
2. Add 230μL Buffer B to the cracking solution and mix well. Centrifuge at 13000rpm for 5min at 4℃.
3. Absorb 600μL supernatant and transfer it to a new 1.5mL EP tube. Add 300μL of anhydrous ethanol into the above solution, shake and mix well, and leave for 5min at room temperature.
4. The above liquid was transferred to the miRNA adsorption column, centrifuged at 12000rpm at 4℃ for 1min, and filtrate was collected.
5. Collect 800μL filtrate into 1.5mL new EP tube, add 300μL isopropyl alcohol, shake and mix well.
6. Transfer the above liquid to the new miRNA adsorption column in two stages. At 12000rpm, centrifuge at 4℃ for 1min and discard the filtrate.
7. Add 700μL bleach solution to the adsorption column and wash it once, centrifuge at 12000rpm for 1min at 4℃, and discard the filtrate.
8. Add 500μL anhydrous ethanol into the adsorption column and wash it once, centrifuge at 12000rpm for 1min at 4℃, then discard the filtrate.
9. The adsorption column was centrifuged empty at 12000rpm for 2min at 4℃ to remove the residual ethanol.

10. The adsorption column was placed into a new 1.5mL EP tube and placed at room temperature for 2min to volatilize the residual ethanol. Add 30μL eluent to the filter element of the adsorption column and let it stand at room temperature for 2min. The eluted miRNA was obtained by centrifugation at 12000rpm at 4℃ for 2min.

**二、Cell sample extraction**

1. Adherent cells: After digestion of cells by pancreatic enzymes, cells were collected by centrifugation, cells were suspended with 100μL 1×PBS, 500μL lysate was added, and the cells were absorbed and mixed until completely dissolved in lysate. Let stand at room temperature for 5min to fully lysate cells.
2. Suspension cells: After direct centrifugation, collect cell precipitation, add 500μL lysate, and mix until the cells are completely dissolved in lysate. Let stand at room temperature for 5min to fully lysate cells.
3. Add 250μL Buffer B to the cracking solution and mix well. Centrifuge at 13000rpm for 5min at 4℃.
4. Absorb 700μL supernatant and transfer it to a new 1.5mL EP tube. Add 300μL of anhydrous ethanol into the above solution, shake and mix well, and leave for 5min at room temperature.
5. Transfer the liquid to miRNA adsorption column A1. Centrifuge at 12000rpm for 1min at 4℃ and collect filtrate.
6. Collect 950μL filtrate into 1.5mL new EP tube, add 400μL isopropyl alcohol, shake and mix well.
7. The above liquid was transferred to miRNA adsorption column A2 in two phases. Centrifuge at 12000rpm for 1min at 4℃, discard filtrate.
8. Add 700μL bleach solution to the adsorption column and wash it once. Centrifuge at 12000rpm for 1min at 4℃, discard filtrate.
9. Add 500μL anhydrous ethanol to the adsorption column and wash it once. Centrifuge at 12000rpm for 1min at 4℃, discard filtrate.
10. The adsorption column was centrifuged empty at 12000rpm for 2min at 4℃ to remove the residual ethanol.
11. The adsorption column was placed into a new 1.5mL EP tube and placed at room temperature for 2min to volatilize the residual ethanol. Add 30μL eluent to the filter element of the adsorption column and let it stand at room temperature for 2min. The eluted miRNA was obtained by centrifugation at 12000rpm at 4℃ for 2min.

**Example:**




**The extracted miRNAs can be used to detect specific primers:**

The quality of miRNA extracted by this kit was tested, including tissue and cell samples. In order to further test whether miRNA extracted by this kit can be specifically applied to fluorescent quantitative PCR, tissue and cell specific primers (m29a-3p, hsa-mi24) were designed for verification. Fluorescence quantitative PCR analysis showed that the dissolution curve was relatively neat, indicating that it was prim-specific amplification. Moreover, the specificity of the melting curve is good and the CT value is close, indicating that this method can obtain the fluorescence quantitative PCR detection of miRNA that can be stably applied to specific primers.

**Related products:**

*R1600 DEPC Treating water*
*R1050 5×RNA Loading Buffer*
*M1010 10×MOPS Buffer*
*R2200 Column extraction of whole blood miRNA kit*
*R2230 Column extraction of plant miRNA kit*