

## Micro RNA Kit (from micro sample) Cat No. ODP420-01

### Kit contents

| Components                 | 50 Preps    |
|----------------------------|-------------|
| Buffer LB1                 | 20mL        |
| Buffer PW                  | 15mL        |
| RNase-free water           | 10mL        |
| Carrier RNA                | 500 $\mu$ L |
| RNase-free spin column CR3 | 50          |
| Collection tubes 2mL       | 50          |
| Handbook                   | 1           |

### Storage

Micro RNA Kit could be stored dry at room temperature (15-25°C) and is stable for 12 months without any diminution in performance and quality. For long term storage, the kit could be stored at 2-8°C. If precipitation occurs under 2-8°C, please place the kit under room temperature.

**Note:** Carrier RNA should be stored at -20°C.

### Introduction

Micro RNA Kit is based on silica membrane technology and special buffer system for RNA extraction from cells and tissues. The spin column, made of new type silica membrane can bind RNA optimally on given salt and pH conditions. Simple centrifugation completely removes contaminants and enzyme inhibitors such as RNase, proteins and divalent cations. Extracted RNA is ready-to-use in downstream applications like restriction analysis, PCR analysis and Southern blotting.

### Precautions

1. Repeated freezing and thawing of stored samples may lead to lower RNA yield.
2. Equilibrate the samples to room temperature.
3. Buffer LB1 uses Guanidine salt. Do not discard solution in bleach such as sodium hypochlorite as Guanidine salts produces hazardous gas when in contact with bleach.

### Materials required but not provided in the kit

1.5mL RNase-free micro centrifuge tubes, Ethanol (96-100%)

### Protocol

**Ensure that Buffer PW have been added with appropriate volume of ethanol (96-100%) as indicated on the bottle and mix thoroughly.**

1. Preparation of samples:
  - a) Adherent cells: Aspirate media and wash cell monolayer with an appropriate amount of PBS. Aspirate PBS and add 200 $\mu$ L of Buffer LB1 directly to culture plate. Lyse cells by gently tapping culture dish and swirling buffer around plate surface for five minutes. Transfer lysate to a RNase-free microcentrifuge tube.
  - b) Suspension cells: Transfer cell suspension to an RNase-free tube (not provided) and centrifuge at no more than 200 x g (~2,000 rpm) for 10 minutes to pellet cells. Carefully decant the supernatant and add 200 $\mu$ L of Buffer LB1 to the pellet. Lyse cells by vortexing for 15 seconds. Ensure that the entire pellet is completely dissolved before proceeding to the next step.

**Note:** For inputs of over 10<sup>5</sup> cells, 5-10 $\mu$ L of media may be left behind with the pellet in order to ensure that the pellet is not dislodged. For inputs of fewer than 10<sup>5</sup> cells, 30-50 $\mu$ L of media may be left behind in order to ensure that the pellet, which could be invisible, is not dislodged.

- c) Tissues:
- Add 200 $\mu$ L of Buffer LB1 to the tissue sample (up to 3mg in most cases) and continue to grind until the sample has been homogenized.  
**Note: An additional Proteinase K (Cat. No.: ORT403, not provided) treatment is required for a larger amount of starting material or fibrous tissue. Add 20 $\mu$ L of Proteinase K to the lysate, and incubate at 55°C for 15 minutes. Vortex the tubes occasionally during incubation**
  - Spin the lysate for 2 minutes at 500 x g (~5,000 rpm) to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube.  
**Note: Volume of Buffer LB1 can be increased if the tissue is hard to lyse.**
- d) Blood: Take fresh blood, and add three volumes of Buffer LB1. Mix thoroughly. (0.75mL Buffer LB1 for 0.25mL whole blood).
- e) Peripheral Blood Mononuclear Cells (PBMC): Isolate PBMC from 5mL whole blood using Ficoll-Hypaque separation solution and density gradient centrifugation. Wash the cells with PBS and transfer the isolated cells to a 1.5mL microcentrifuge tube. Add 1mL Buffer LB1 to the tube and mix the contents by repeated pipetting.
2. Add 10 $\mu$ L of Carrier RNA and mix the contents by vortexing for 10 seconds. Incubate at room temperature for 3 minutes.
  3. Add 60 $\mu$ L of 96 – 100% ethanol to every 100 $\mu$ L of lysate/supernatant of samples. Mix by vortexing for 30 seconds.
  4. Pipette the mixture into the spin column CR3 (in a 2mL collection tube) and centrifuge at 10,000 rpm for 1 minute. Discard the flow-through and place the spin column back to the collection tube.
  5. Add 700 $\mu$ L Buffer PW to spin column CR3 and centrifuge at 1000 x g (~10,000 rpm) for 1 minute. Discard the flow-through and place the spin column back to the collection tube.
  6. Repeat the step.
  7. Re-centrifuge the column placed in collection tube at 1000 x g (~10,000 rpm) for 2 minutes and discard the flow-through.
  8. Place the spin column CR3 to a new clean 1.5mL micro-centrifuge tube and open the lid of the spin column CR3 and incubate the assembly at room temperature (15-25°C) for 2 minutes to dry membrane completely.  
**Note: Residual ethanol from Buffer PW may inhibit subsequent enzymatic reactions.**
  9. Pipette 50 $\mu$ L RNase-free water directly to the center of membrane. Incubate at room temperature (15-25°C) for 5 minutes. Then centrifuge at 1000 x g (~10,000 rpm) for 1 minute.  
**Note: The volume of elution buffer should not be less than 30 $\mu$ L, or it may affect recovery efficiency. To obtain higher productivity, add the solution gained from step 8 to the center of membrane again, let the spin column stand for 1 minute, and then centrifuge. Extracted RNA should be stored at -80°C.**