

## MolPure® Cell /Tissue Total RNA Kit

### Product description

MolPure® Cell /Tissue Total RNA Kit was performed by using MolPure® DNA removing / RNA binding column technology and new solution systems suitable for extracting high purity and quality total RNA from a variety of fresh or frozen animal tissues or cultured cells. The extraction process does not require toxic phenol, chloroform,  $\beta$ -mercaptoethanol, or precipitation of isopropanol and ethanol. Easy to operate, 15 min to complete the animal tissue or cells (10-30 mg animal tissue or  $(1-10) \times 10^6$  Animal cells) of total RNA extraction. The total RNA is of high purity and can be used in various molecular biology experiments including RT-PCR, qPCR, molecular cloning and RNase protection analysis.

### Specifications

Cat.No.	19221ES08 / 19221ES50 /
Size	5 T / 50 T

### Components

Components No.	Name	19221ES 08	19221ES 50
19221-A	MolPure® DNA removing /RNA binding Column A2	10	100
19221-B	2 mL Collection Tube A 2	10	100
19221-C	Lysate buffer LB (LB Buffer A 2)	3 mL	30 mL
19221-D	Deproteinized buffer PL (PL Buffer A 2)	4 mL	40 mL
19221-E	Binding buffer BD*(BD Buffer A 2*)	1 mL	10 mL
19221-F	Wash Buffer W*(Wash Buffer A 2*)	1.3 mL	13 mL
19221-G	RNase -free H2O	1 mL	5 mL

### Storage

This product should be Store at room temperature, protected from light, for 24 months. 2-8°C lasts longer.

### Instructions Preparation before the experiment

1. Self-provided equipment and reagents : desktop centrifuge, water bath or metal bath, 1.5 mL RNase- free centrifuge tube, liquid nitrogen, absolute ethanol, etc.
2. This kit can inhibit RNase activity without low temperature centrifugation, and all centrifugation steps are performed at room temperature.
3. Before the first use, Add the labeled amount of absolute ethanol (5 T / 50 T with 2.4 mL / 24 mL absolute ethanol) in the **binding buffer BD\* (19221-E)**, mix thoroughly and mark.
4. Before the first use, Add the labeled amount of absolute ethanol (5 T / 50 T and 50, add 5.2 mL / 52 mL absolute ethanol) in the **Wash Buffer W\* (19221-F)**, mix thoroughly and mark.

### Method of operation

## 1. Sample preprocessing

- ✧ **For animal tissues** : 350  $\mu$  L of **Lysate buffer LB** was added to fresh tissue (<20 mg) and the tissue was ground evenly with a glass homogenizer or electric homogenizer. If used liquid nitrogen Grinding, need to grind into a fine powder and then add the corresponding amount of **Lysate buffer LB**, violent shock for 20s, fully mixed.
- ✧ **For adherent cells** : no digestion, direct lysis, or after centrifugation, add 350  $\mu$  L of **Lysate buffer LB** (<5 $\times$ 10<sup>6</sup> Cells), mix them repeatedly with a pipete (until no cell mass is visible).
- ✧ **For suspended cells** : Cells were collected by direct centrifugation with 350  $\mu$  L of **Lysate buffer LB** (<5 $\times$ 10<sup>6</sup> Cells), mix them repeatedly with a pipete (until no cells are visible).

[Note]: 600  $\mu$  L of **Lysate buffer LB** was added to 20-30 mg tissue

[Note]: 600  $\mu$  L of **Lysate buffer LB** was added to (5-10) $\times$ 10<sup>6</sup> cells

## 2. RNA extraction

1. Add the treated homogenate to the **MolPure<sup>®</sup> DNA removing /RNA binding Column A2** (column in a 2 mL Collection Tube) and centrifuge at 13,000 rpm for 1 min to collect the filtrate containing RNA.
2. Accurately estimate the equal volume of **Binding buffer BD\*** in the filtrate volume\* (Please confirm that the absolute ethanol has been added first!), Immediately blow gently and mix well.
3. All the above mixture was added to a new **MolPure<sup>®</sup> DNA removing /RNA binding Column A2**, centrifuged at 13,000 rpm for 30s, and the filtrate was discarded.
4. 700  $\mu$  L of **Deproteinized buffer PL**, centrifuged at room temperature for 30s, then 30s at 13,000 rpm, the filtrate was discarded and the **MolPure<sup>®</sup> DNA removing /RNA binding Column A2** was put back into a 2 mL Collection Tube.
5. Add 500  $\mu$  L of **Wash Buffer W\*** (Please confirm that the absolute ethanol has been added first!), 13,000 rpm for 30s and the filtrate was discarded.
6. Repeat step 5 to return the **MolPure<sup>®</sup> DNA removing /RNA binding Column A2** into the 2 mL collection tube.
7. Empty column was centrifuged at 13,000 rpm for 2 min to remove the residual **Wash Buffer W\***.
8. The **MolPure<sup>®</sup> DNA removing /RNA binding Column A2** was placed into a new 1.5 mL RNase-free centrifuge tube, and 30-50  $\mu$  L RNase-free H<sub>2</sub>O was added to the center of the membrane, and placed at room temperature at 1 min and then centrifuged at 13,000 rpm for 1 min to collect the filtrate, the RNA solution. RNA can be stored at -80°C.

[Note]: The yield can be increased by the following ways: ① 65°C preheating RNase-free H<sub>2</sub>O; ② loaded the RNA filtrate onto the column again and left at room temperature for 1 min before elution.

## Notes

1. Avoid volatilization, oxidation and pH changes caused by the reagent exposed to air for a long time. Each solution should be closed in time after use.
2. Pay attention to observe whether each solution has precipitation or turbidity (especially when the room temperature is low temperature in winter), can be 37°C warm bath resolution to the solution clarification, to avoid affecting the use effect.
3. For your safety and health, please wear laboratory clothes and wear disposable gloves.
4. This product is used for scientific research only!