

### **RNA Later**

## Composition

Cat. No.	4007020	4007100	4007500
RNA Later	20 ml	100 ml	500 ml
Instructions	1	1	1

### **Storage**

RNA Later can be stored for more than 2 years at room temperature (0-30°C) without changes in performance. Low temperature storage may produce crystal precipitation, incubate at 37°C to completely dissolve precipitation before use.

# **Technical Support**

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#### Introduction

RNA Later is a liquid, non-toxic tissue preservation reagent. It can quickly penetrate tissue cells, protect non-frozen cell RNA in situ by highly inhibiting RNase activity, and make it more suitable for tissue gene expression profile analysis. Once the tissue blocks are obtained, they can be quickly immersed in the RNA Later, so that the sample does not have to be processed immediately or frozen in liquid nitrogen. RNA Later can be applied to a wide range of vertebrate samples, including brain, heart, kidney, spleen, liver, lung, and thymus.

## **RNA Later Dosage for Different Tissue Samples**

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Samples	Sample Amount (mg)	RNA Later Dosage (ml)
Renal	100-500	1-5
Spleen	100-300	1-3
Lung	100-300	1-3
Heart	100-170	1-1.7
Liver	100-1000	1-10

Fresh non-frozen tissues were immersed in RNA Later at a ratio of 1:10 and stored at 37°C for 1 day, room temperature for 1 week and 4°C for at least 1 month; The tissues can be stored at -20°C or -80°C for a long time after soaking at 4°C overnight. Tissues preserved in RNA Later can be frozen and thawed repeatedly for at least 20 times.

#### Note

- 1. RNA Later should only be used on fresh tissue, and tissue should not be frozen before being immersed in the RNA Later.
- 2. The maximum thickness of either side of the tissue sample should not be > 0.5 cm, and then the tissue block should be stored in the RNA Later with 10 times of the volume. If the thickness of the tissue sample is too large, the slow penetration of the RNA storage solution into the tissue sample will cause RNA degradation. Therefore, when the tissue sample thickness exceeds 0.5 cm, the tissue should be simply chopped and then stored in the RNA Later.



### **Protocol**

- 1. Estimate the tissue weight to be added to the RNA Later before cutting the tissue and determine the RNA Later dosage to be used based on the amount added to at least 10 times the tissue volume (example: 100 mg tissue requires 1 ml RNA Later).
- 2. Place the tissue into the RNA Later after cutting. If the tissue sample is too large, the maximum thickness on either side should not > 0.5 cm.
- \* To be fully effective at protecting a tissue sample, the tissue sample should be fully immersed in the RNA Later, and the maximum thickness of either side of the tissue sample should not >0.5 cm.
- 3. Tissue samples stored in RNA Later can be stored at 4°C for at least 1 month, at room temperature for 1 week, and at 37°C for 1 day. For long-term storage at -20°C or -80°C, the samples were first soaked overnight in the sample storage solution at 4°C, and then the tissues were removed from the RNA Later and stored at -20°C or -80°C for long-term storage. The samples can then be thawed and refrozen at room temperature without affecting the quality and quantity of RNA.
- \* Tissue samples are recommended to be stored at low temperature in RNA Later (at least 1 month at 4°C, long-term storage at -20°C or -80°C), not at 37°C or room temperature. Tissues frozen at -20°C or -80°C can be frozen and thawed repeatedly for at least 20 times without affecting RNA extraction. If the tissue stored in the RNA Later needs to be transported over long distances, it is necessary to ensure that the tissue is fully immersed in the RNA Later during transportation.
- 4. After the sample is removed from the RNA Later, RNA can be extracted directly with the RNA extraction kit (Trizol, Simzol, Ultra-Pure Total RNA Extraction Kit or Animal Tissue Total RNA Kit).

#### Other applications:

- Many plant tissues can be directly immersed in the RNA Later. Some plants have a thick layer of
  wax on the surface of the leaves. The wax layer needs to be destroyed before the RNA Later can
  fully penetrate the tissue.
- 2. For the operation of tissue culture cells, the cells were first precipitated, washed once with PBS, then suspended with a small amount of PBS, added 2-3 times the amount of cell suspension fluid to preserve the RNA Later. Subsequent RNA extraction can be performed by centrifuge after removing the RNA Later or add RNA extraction reagents without removing the RNA Later for direct extraction.
- 3. **Centrifugal method:** Centrifuge the precipitated cells and remove the RNA Later. Because the concentration of the medium in the RNA Later is higher than that of typical cell culture medium, the cells in the RNA Later cannot be precipitated with the centrifugal force normally used to precipitate living cells. (HeLa cells need about 3000×g, but other cells may not tolerate this speed, or they may need a greater centrifugal force.)
- 4. **Direct extraction method:** This is done by adding 10 times the volume of RNA extraction reagents to the cell mixture.
- 5. For the operation of bacteria, the bacteria are collected by centrifuge first, washed with PBS once, then suspended with a small amount of PBS, and stored in the RNA Later with 2-3 times the volume of bacterial suspended liquid. The subsequent RNA extraction was carried out in accordance with the extraction steps of tissue cells. *E. coli* was kept in the RNA Later at 4°C for 1 month and was still intact, producing non-degraded RNA.
- 6. For the preservation of white blood cells in whole blood, white blood cells need to be separated from red blood cells and serum, and treated as tissue culture cells, so that white blood cells can be effectively preserved in the RNA Later. Do not store the RNA in whole blood, plasma, or serum in the RNA Later, because their protein content is too high, and they are easy to form insoluble precipitates after mixing with the RNA Later.