

## Sample Stabilization and Storage in KeepRNA Solution

KeepRNA is a non-toxic aqueous solution that rapidly penetrates cells and tissues, protecting RNA from degradation. The solution is a complete analog of RNAlater™. KeepRNA is used for rapid stabilization and storage of cellular RNA in tissues and cell cultures. The solution allows for the storage and transport of samples for up to 7 days at room temperature or about 1 month at 4 °C, without resorting to laborious rapid RNA isolation or pre-freezing of tissue.

Cells and tissues stored in KeepRNA can be used for subsequent isolation of mRNA and total RNA, as well as for histological and immunochemical methods. KeepRNA is compatible with all standard RNA isolation methods and kits. RNA isolated from tissue fragments after storage in KeepRNA retains its qualitative and quantitative composition and can be used for any molecular biology applications.

In addition to RNA, DNA and proteins can also be extracted from samples stabilized with KeepRNA. It should be noted that proteins denature under the action of the KeepRNA solution. Therefore, the extracted proteins are suitable only for methods that do not require the native conformation, such as Western blotting and two-dimensional electrophoresis.

## Samples Compatible with KeepRNA

KeepRNA can be used to preserve RNA in most animal and plant tissues, cultured cells, bacteria, and yeast. Exceptions include poorly permeable tissues, such as waxy plant tissues and bone.

## Sample Preparation

### Animal Tissue

The KeepRNA solution does not disrupt tissue structure, allowing the impregnated sample to be removed from the solution, divided into portions for analysis, and, if necessary, returned to the reagent for further storage. Small organs (e.g., the liver, kidney, or spleen) from laboratory animals can be stored in the solution whole, without prior dissection.

### Blood

KeepRNA is only effective for isolated leukocytes (purified from erythrocytes and serum), which should be processed as a cell culture. Do not use KeepRNA for whole blood, plasma, or serum; this will result in insoluble precipitate formation.

### Plant tissue

For tissues with natural barriers (e.g., a waxy cuticle layer), additional pretreatment may be required to ensure adequate penetration of the KeepRNA solution.

At the same time, many plant samples can be immersed whole in the KeepRNA solution.

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## Cell cultures

Pellet the cells according to your laboratory's established protocol. Remove the supernatant and add 5-10 volumes of KeepRNA solution. If necessary, cells can be washed with phosphate-buffered saline (PBS) before resuspension in KeepRNA.

## Bacterial cultures

KeepRNA has a bacteriostatic effect, preventing bacterial multiplication while maintaining the integrity of their cells. Sample storage is recommended at 4 °C.

## Sample Storage

### Tissue samples

1. Collect the tissue samples immediately after extraction. For optimal reagent penetration, cut the tissue into small fragments no more than 0.5 cm thick in any dimension.
2. Place the tissue fragments in a tube with 5 volumes of KeepRNA solution. For example, a 0.5 g tissue sample should be wholly immersed in at least 2.5 mL of KeepRNA. Small organs (e.g., a rat kidney) can be placed whole in the solution.
3. KeepRNA solution provides flexible storage conditions depending on the required storage time:
  - Up to 1 day: at 37 °C
  - Up to 1 week: at 25 °C
  - Up to 1 month and longer: at 4 °C

### Cell Cultures

1. Collect and sediment cells according to standard laboratory protocol.
2. Remove the supernatant and wash the cell pellet with a small volume of PBS.
3. Mix the resuspended cells with 5–10 volumes of KeepRNA.
4. Store the sample following the same temperature recommendations as for native tissue samples.

## RNA Isolation

*Important!* Before starting the RNA isolation procedure, the KeepRNA solution must be completely removed from the sample.

1. Remove KeepRNA from the sample. RNase inactivation is reversible, so do not wash the sample with water.
2. For tissue: blot the tissue with a clean tissue to remove excess KeepRNA.
3. For cells: sediment the cells and remove the supernatant. Since KeepRNA has a higher density than regular medium, increased centrifugation speed may be required (e.g., 5,000× *g*). Alternatively, before centrifugation, you can dilute KeepRNA with an equal volume of cold PBS (4 °C).
4. Lyse and homogenize the sample. Add the RNA isolation lysis solution directly to the sample and immediately homogenize the tissue. For harder tissues (e.g., bone), pre-grinding in liquid nitrogen may be necessary.
5. Isolate RNA using a standard protocol. The homogenized sample can be processed using most standard RNA isolation methods.

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