

Protocol for RNA Stabilization with RNA^{later} RNA Stabilization Reagent

This protocol describes the procedure for storing human and animal tissues in RNA^{later} RNA Stabilization Reagent. For RNA isolation using RNeasy Protect Kits or RNeasy Kits, see the *RNeasy Mini Handbook* and the *RNeasy Midi/Maxi Handbook*.

Important notes before starting

- If using RNA^{later} TissueProtect Tubes or RNA^{later} RNA Stabilization Reagent for the first time, read “Important Points before Using RNA^{later} TissueProtect Tubes and RNA^{later} RNA Stabilization Reagent” on page 7.
- It is important to use sufficient volumes of RNA^{later} RNA Stabilization Reagent to effectively preserve the RNA in tissues. Using too little reagent will lead to degradation of RNA in the tissues during storage. Read “Determining the volume of RNA^{later} RNA Stabilization Reagent for RNA stabilization in animal tissues” on page 7.
- The tissue must be cut into slices less than 0.5 cm thick. If the tissue slice is thicker than 0.5 cm, it will need to be cut into thinner slices before storing in RNA^{later} RNA Stabilization Reagent (see step 3 in the protocol, and read “Maximum tissue size for effective stabilization” on page 7). If tissue slices are thicker than 0.5 cm, diffusion of the reagent into the sample may be too slow for reliable RNA stabilization in the interior. RNA degradation will occur in tissue slices that are too thick.
- Only fresh, unfrozen samples can be stabilized using RNA^{later} RNA Stabilization Reagent. Previously frozen tissue samples thaw too slowly in the reagent, thus preventing it from diffusing into the tissue quickly enough before the RNA begins to degrade.
- RNA is not protected after harvesting until the sample is treated with RNA^{later} RNA Stabilization Reagent. Samples should be submerged in the appropriate volume of RNA^{later} RNA Stabilization Reagent **immediately** after harvesting the material (see steps 1–3 in the protocol).
- RNA^{later} RNA Stabilization Reagent may form a precipitate when stored below room temperature (15–25°C). The precipitate can be redissolved by heating to 37°C with agitation. Redissolve any precipitate before using.
- RNA^{later} TissueProtect Tubes are for single use only. Do not reuse the tubes.

1. **Before excising the tissue sample, estimate the volume (or weight) of the tissue piece to be stabilized in RNA^{later} RNA Stabilization Reagent.**
2. **Determine the appropriate volume of RNA^{later} RNA Stabilization Reagent needed for preserving the tissue to be used. A minimum of 10 volumes of RNA^{later} RNA Stabilization Reagent (or approximately 10 μ l/1 mg of tissue) is required. Calculate the correct amount, and choose the appropriate RNA^{later} TissueProtect Tube.**

The small RNA^{later} TissueProtect Tubes are designed for stabilization of up to 150 mg of tissue; the large RNA^{later} TissueProtect Tubes can be used to efficiently stabilize up to 500 mg of tissue.

If the tissue samples are to be transported in RNA^{later} RNA Stabilization Reagent ensure that the tissue remains submerged in the liquid during transport. Ensure that the tubes remain upright during transport.

Note: The tissue should be placed in **at least 10 volumes of RNA^{later} RNA Stabilization Reagent (or 10 μ l reagent per 1 mg tissue)**. Smaller volumes will lead to RNA degradation during storage. See "Determining the volume of RNA^{later} RNA Stabilization Reagent for RNA stabilization in animal tissues" on page 7.

3. **Excise the tissue sample from the animal, and cut it into slices less than 0.5 cm thick. Perform this step as quickly as possible. Then proceed immediately with step 4.**

If the tissue is already thinner than 0.5 cm (in at least one dimension), it does not need to be cut into smaller pieces. Proceed immediately with step 4.

Note: If tissue slices are thicker than 0.5 cm, diffusion of the reagent into the sample will be too slow for reliable RNA stabilization in the interior. RNA degradation will occur in tissue slices that are too thick. Simply cut larger tissue pieces prior to stabilization into slices less than 0.5 cm thick. The slices can be any convenient size so long as one dimension of the sample is <0.5 cm. See "Maximum tissue size for effective stabilization" on page 7.

RNA in tissues is not protected after harvesting until the sample is treated with RNA^{later} RNA Stabilization Reagent. Submerge the sample in a sufficient volume of the reagent immediately after slicing.

4. **Completely submerge the tissue piece(s) in the RNA^{later} RNA Stabilization Reagent.**

Note: RNA in tissues is not protected after harvesting until the sample is treated with RNA^{later} RNA Stabilization Reagent. Samples should be submerged in the appropriate volume of the reagent **immediately** after harvesting the material.

If the tissue samples are to be transported in RNA^{later} RNA Stabilization Reagent, ensure that the tissue remains submerged during transport. Ensure that the tubes remain upright during transport.

5. Store the tissue submerged in RNA $later$ RNA Stabilization Reagent for up to 4 weeks at 2–8°C, up to 7 days at 18–25°C, or up to 1 day at 37°C.

For archival storage at –20°C, first incubate the sample overnight in the reagent at 2–8°C. Then transfer the tissue, in the reagent, to –20°C for storage.

For archival storage at –80°C, first incubate the sample overnight in the reagent at 2–8°C. Then remove the tissue from the RNA $later$ RNA Stabilization Reagent, and transfer it to –80°C for storage.

Samples stored in RNA $later$ RNA Stabilization Reagent at –20°C will not freeze. The low temperature may cause the formation of crystals or a precipitate in the storage solution. This will not affect subsequent RNA isolation. There is no need to redissolve the precipitate.

Processed samples stored at –20°C or –80°C can be thawed at room temperature and frozen again for up to 20 freeze–thaw cycles without affecting RNA quality or yield.

Note: If available, lower temperatures are recommended for longer storage (e.g., 2–8°C for up to 4 weeks instead of 37°C or room temperature; –20°C or –80°C for longer storage).