Top Ten Ways to Improve Your RNA Isolation

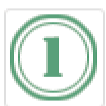
Effective preparation of RNA is a fundamental technique that is required for a wide variety of exciting and information-rich analysis techniques including next-generation sequencing, reverse transcription qPCR (RT-qPCR), northern blot analysis, and cRNA production. The fidelity of transcriptome representation, and the quality and quantity of recovered RNA, will significantly impact the resulting analysis.

A variety of factors during sample collection, processing, and storage can negatively impact results and should be considered. These factors broadly fall into three categories:

- Stabilization of a sample before processing
- Recovery of RNA during manipulations
- Exposure of unprotected samples to RNases

This article highlights laboratory best practices for RNA preparation and describes 10 ways to improve RNA purification.

1. Immediately Inactivate Endogenous, Intracellular RNases
2. Use Proper Cell or Tissue Storage Conditions
3. Thoroughly Homogenize Samples
4. Pretreat Homogenate Before RNA Isolation
5. Choose the Best RNA Isolation Method
6. DNase Treatment
7. Reduce Exposure to Environmental RNases
8. Proper Precipitation
9. Resuspension
10. Storage



Immediately Inactivate Endogenous, Intracellular RNases

Endogenous RNases must be inactivated immediately upon tissue harvesting and cell death to prevent RNA degradation.

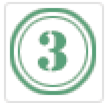
There are 3 effective methods to accomplish this:

- Homogenize samples immediately after harvesting in a chaotropic-based cell lysis solution (e.g., containing guanidinium).

- Flash freeze samples in liquid nitrogen. In order to inactivate RNase by flash freezing, it is important that tissue pieces be small enough to freeze almost immediately upon immersion in liquid nitrogen.
- Place samples in RNA*later*® Tissue Collection: RNA Stabilization Solution, an aqueous, nontoxic collection reagent that stabilizes and protects cellular RNA in intact, unfrozen tissue and cell samples (see #2 below). It is essential that tissue samples be in thin pieces (0.5 cm) so that the RNA*later*® can quickly permeate the tissue before RNases destroy the RNA.

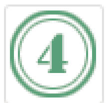
Use Proper Cell or Tissue Storage Conditions

When samples have been flash-frozen they must be stored at –80°C and never allowed to thaw. Even brief thawing prior to homogenization in a guanidinium-based lysis solution can result in RNA degradation and loss. Flash-frozen tissues should be homogenized in chaotropic lysis buffer directly from the frozen state, or ground or pulverized at cryogenic temperatures prior to homogenization in a lysis solution.



Thoroughly Homogenize Samples

Thorough homogenization of cells or tissues is an essential step in RNA isolation that prevents both RNA loss and RNA degradation. The method of homogenization should be tailored to the cell or tissue type. Whereas most cultured cells can be homogenized by simply vortexing in a cell lysis solution, animal tissues, plant tissues, yeast, and bacteria often require more rigorous methods of disruption. For example, bacterial cell walls may require enzymatic digestion to achieve thorough cell lysis and maximum recovery of RNA.



Pretreat Homogenate Before RNA Isolation

Additional treatments are needed for some samples after homogenization and before RNA isolation. Lysates made from tissues high in fat, like brain and adipose tissue, should be extracted with chloroform to remove lipids and increase RNA yields. Many plant tissues are high in polyphenolics and polysaccharides that can decrease RNA quality and yield. Pretreatment of the lysate with the Plant RNA Isolation Aid removes these troublesome compounds.

Choose the Best RNA Isolation Method

The wide variety of RNA isolation methods available can make it difficult to decide which one to use. The easiest and safest methods available are column-based methods like the PureLink® RNA Mini Kit or PureLink® Pro 96 Kit. Due to ease of handling, these procedures are ideal for working with multiple samples. The MagMAX™-96 Total RNA Isolation Kit and MagMAX™-96 for Microarrays Total RNA Isolation Kit utilize a paramagnetic particle approach, which is easy to automate and avoids clogging that can occur

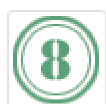
with some tissues. When working with difficult tissues, for example ones that are high in nucleases (pancreas) or fat (brain and adipose tissue), a more rigorous, phenol-based RNA isolation method using TRIzol® Reagent is recommended. For the ultimate performance and convenience in preparing tissue culture cells for analysis, we recommend the Cells-to-CT™ Kit family.

DNase Treatment

When the RNA will be used for RT-PCR, we recommend treating it with DNase to remove residual contaminating DNA. DNase treatment is also a good idea when isolating RNA from tissues that are high in DNA, like spleen. The PureLink® DNase Set has been optimized for effective on-column removal of DNA from RNA, and is supplied with reagents necessary for treating samples. Samples can also be treated with TURBO DNA-free™ DNase treatment and Removal Reagents or DNase I, Amplification Grade to remove contaminating DNA from RNA previously purified by any method. Both products offer high-quality DNase I plus an optimized reaction buffer, providing a quick and easy way to remove the DNase after the treatment without using organic solvents or risking a heat treatment.

Reduce Exposure to Environmental RNases

To isolate intact, high-quality RNA, it is essential that RNases are not introduced into RNA preparations once they are no longer protected by strong protein denaturants such as a chaotropic lysis solution or phenol derivatives. RNases are found almost everywhere, and it is essential that any item that could contact the purified RNA be RNase-free. All surfaces, including pipettors, benchtops, glassware, and gel equipment, should be decontaminated with a surface decontamination solution like RNaseZap® solution or RNaseZap® Wipes. RNase-free tips, tubes, and solutions should always be used and gloves should be changed frequently.



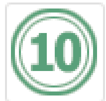
Proper Precipitation

Purified RNA may need to be concentrated by precipitation for downstream applications. Precipitating RNA with alcohol (ethanol or isopropanol) requires a minimum concentration of monovalent cations (for example, 0.2 M Na^+, K^+, and 0.5 M NH_4^+). After the salt concentration has been adjusted, the RNA may be precipitated by adding 2.5 volumes of ethanol or 1 volume of isopropanol and mixing thoroughly, followed by chilling for at least 15 minutes at –20°C. After chilling, spin the RNA at 12,000 x g for 10 minutes and carefully remove the supernatant. While isopropanol is somewhat less efficient at precipitating RNA, isopropanol in the presence of NH_4^+ is better than ethanol at keeping free nucleotides in solution, separating them from precipitated RNA. For quantitative recovery of low concentrations of RNA (ng/mL), an inert coprecipitant (e.g., glycogen, yeast RNA, or linear acrylamide) should be used. Linear acrylamide and DNase-treated glycogen are the coprecipitants of choice when the RNA will be used in RT-qPCR because they do not contain contaminating DNA. Yeast RNA and untreated glycogen could introduce nucleic acid contamination into samples, potentially skewing RT-qPCR results. After precipitation and

removal of the supernatant, avoid complete drying of the RNA pellet because it can make RNA difficult to resuspend.

Resuspension

The final step in many RNA isolation procedures is to resuspend the purified RNA pellet. The three ideal qualities of a resuspension solution are that it be RNase-free, have a slightly acidic pH (pH 6–7), and incorporate a chelating agent to protect against RNA degradation by introduced RNases (THE RNA Storage Solution meets all of these criteria). To aid solubilization, the RNA pellet should be incubated in resuspension solution at 65°C for 5 minutes with intermittent gentle vortexing.



Storage

For short-term storage, resuspended RNA should be stored at –20°C; for long-term storage, it should be stored at –80°C. Although RNA resuspended in water or buffer can be stored at –80°C, RNA is most stable in an NH_4OAc/ethanol precipitation mixture at –80°C. We recommend aliquoting RNA solutions into several tubes. This will both prevent damage to the RNA from successive freeze-thaw events and help to prevent accidental RNase contamination.