

Guidelines for Handling RNA

A common reason why groups working with RNA fail to obtain results is because of contamination by RNAses, which can rapidly degrade your costly sample into a mixture of nucleoside monophosphates. Several simple precautions can safeguard your product.

- Always use sterilized water, preferably water recently treated with DEPC (diethyl pyrocarbonate). DEPC reacts with amines, inactivating most enzymes.

This treatment is very simple. Just add 100 µl of DEPC per liter of high-quality water (HPLC grade works well for this purpose).

Stir the solution for several hours, preferably overnight. Remove excess DEPC (very important) by either heating the solution to 95°C for three hours or autoclaving twice.

- Prepare all of your buffers using water sterilized in this fashion.
- Most freshly opened bottles of commercial HPLC grade water are already RNase free.
- However, always test untreated water (or old treated water) for nucleases by incubating sample RNA in the water at 37°C for at least 3 hours and then run a gel to detect degradation. Don't forget to run a control lane to make sure your sample RNA isn't already degraded!
- Never touch any surface that will come in contact with RNA. RNAses are ubiquitous and are readily transferred from your fingertips.
- The diligent use of gloves is recommended, but must not be expected to prevent RNase contamination altogether. Has that pipet, or anything else you are using ever been touched by someone, who was not wearing gloves? Think about it!
- Always use baked glassware (250°C + for more than 4 hours) and sterile plastic ware. RNAses can survive autoclaving, so do not depend on items sterilized in such a manner to be RNase-free.
- On the other hand, freshly opened bags of non-sterilized plastic ware, such as tubes, are quite safe – as long as you do not contaminate them yourself. These items are produced on machines that are not touched since initial set up, and automatically and immediately bagged afterwards.

Follow this advice and your RNA should remain intact!