



## General Remarks on Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, all plasticware or glassware should be treated to eliminate possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the isolation procedure. In order to create and maintain an RNase-free environment, the following precautions should be followed while working with RNA.

### General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep isolated RNA on ice when aliquots are pipetted for downstream applications.

### Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended when working with RNA. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.

### Non-disposable plasticware

Non-disposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water (see "Solutions", page 48). Alternatively, chloroform-resistant plasticware can be rinsed with chloroform to inactivate RNases.

### Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent, thoroughly rinsed, and oven baked at 240°C for at least 4 hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC\* (diethyl pyrocarbonate). Fill glassware with 0.1% DEPC (0.1% in water), incubate overnight (12 hours) at 37°C, and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.

\* DEPC is a suspected carcinogen and should be handled with great care. Wear gloves and use a fume hood when using this chemical.



## Electrophoresis tanks

Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS), thoroughly rinsed with RNase-free water, and then rinsed with ethanol\* and allowed to dry.

## Solutions

Solutions (water and other solutions) should be treated with 0.1% DEPC.† DEPC is a strong, but not absolute, inhibitor of RNases that works by covalently modifying RNases.

\* Plastics used for some electrophoresis tanks are not resistant to ethanol. Take proper care and check the supplier's instructions.

† DEPC is a suspected carcinogen and should be handled with great care. Wear gloves and use a fume hood when using this chemical.

## Protocol 1. Preparation of RNase-free solutions

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1. Add 0.1 ml DEPC to 100 ml of the solution to be treated. Shake vigorously to bring the DEPC into solution.
2. Incubate for 12 h at 37°C.
3. Autoclave for 15 min to remove any trace of DEPC.

**Tip** DEPC will react with primary amines and cannot be used directly to treat Tris buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO<sub>2</sub>. When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer.

**Tip** Trace amounts of DEPC will modify purine residues in RNA by carboxymethylation. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 min. Carboxymethylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified.

**Tip** RNeasy® buffers from QIAGEN are guaranteed RNase-free without using DEPC treatment and are therefore free of any DEPC contamination.