

Materials needed

Diethyl pyrocarbonate (DEPC)

0.1N NaOH/0.1% EDTA solution

RNase is a very difficult enzyme to remove/denature. Autoclaving is not enough, as RNase rapidly refolds upon cooling. RNase must be destroyed prior to RNA handling. Keep in mind that RNase is everywhere, and sterile lab technique is a must!

Microcentrifuge treatment

Plastic tubes can not be autoclaved for a time sufficient to remove all residual DEPC, so don't use it!

- Fill all tubes with 0.1N NaOH/0.1% EDTA
- Incubate at room temperature overnight
- Empty contents of tube. Rinse thoroughly with DEPC-treated nuclease-free water
- Allow to dry, close, and store in a sealed container

Buffer treatment

- Add 0.05% (v/v) DEPC to HOH or buffer solution.
- Incubate at room temperature overnight
- Autoclave on 30 min liquid cycle to remove residual DEPC

** Tris is incompatible with DEPC. To make nuclease-free Tris, DEPC-treat HOH, add Tris, pH, then sterile filter **

Pipetter treatment

- Spray RnaseZap liberally on pipetter exteriors; immediately wipe clean with paper towel

This protocol borrows heavily from:

- Various Ambion RNA handling protocols/technical notes
- Removing RNase from plastic containers:
<http://www.coleparmer.com/techinfo/techinfo.asp?htmlfile=RNaseRemove.htm&ID=642> (If link is dead, search under “rnase removal chart techniques”)