Preparation of solutions and equipment for isolation of RNA

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Equipment and reagents

- Standard molecular biology equipment and reagents
- Oven
- NaOH
- Diethyl pyrocarbonate (DEPC)

Method

1. Standard procedures[a] to minimize RNase contamination are used for all solutions and glassware. Whenever possible, disposable sterile plasticware is used for any of the steps prior to the RNA being converted to single-stranded cDNA.
2. To minimize RNase contamination, soak glassware with 0.1 M NaOH for 1 h at room temperature, followed by extensive rinsing with diethyl pyrocarbonate (DEPC) treated double distilled, filter sterilized H_2O (ddH_2O). The glassware is then sterilized by autoclaving and baked in an oven at 160 °C overnight.
3. Non-disposable plasticware such as horizontal gel electrophoresis units and centrifuge tubes are also treated with 0.1 M NaOH and rinsed with DEPC treated ddH_2O. Autoclave and bake disposable tubes and Pipetteman tips in an 80 °C oven overnight.
4. Solutions and ddH_2O are treated to eliminate RNase activity by adding DEPC to a final concentration of 0.05% (v/v), stirring the solution at room temperature for 12–16 h, and then autoclaving the solutions to eliminate DEPC which will inhibit downstream enzymatic reactions.

Notes

a Sambrook J., Fritsch E.F., and Maniatis T. *Molecular Cloning: A Laboratory Handbook*, 2nd edn. Cold Spring Harbour Laboratory, Cold Spring Harbour NY

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