

Total RNA Preparation:Phenol/Freeze RNA Prep
Adapted from : Schmitt et al. Nucleic Acids Res 1990;18:3091

Solutions

AE Buffer

50 mM Na acetate pH 5.3
10 mM EDTA

10% SDS

Phenol equilibrated with AE buffer
Phenol:chloroform:isoamyl alcohol 25:24:1
100% and 80% ETOH

- 1] Resuspend yeast cells in 400 ul AE buffer
- 2] Add 40 ul 10% SDS
- 3] Add equal volume phenol
- 4] Vortex
- 5] Incubate at 65C for 4 minutes
- 6] Rapidly chill in ETOH dry ice bath until phenol crystalizes
- 7] Centrifuge top speed for 2 minutes at room temp
- 8] Transfer aqueous phase to new tube
- 9] Add equal volume phenol/chloroform/isoamyl, vortex, centrifuge top speed 5 minutes at room temp
- 11] Transfer aqueous phase to new tube
- 12] Add 1/10 volume 3 M Na acetate
- 13] Add 2.5 volume 100% ETOH
- 14] Centrifuge top speed 5 minutes
- 15] Decant and wash with 80% ETOH
- 16] Air dry pellet (do not allow to dry completely)
- 17] Resuspend in nominal DEPC H2O