RNA isolation (Koshland lab protocol)

Volumes and weights are for 10 ml cultures (1-2 x 10^7 cells/ml).

1. Spin down cells, decant, and resuspend in 0.2 ml extraction buffer with SDS and transfer to a 1.5 ml eppendorf tube. The cells can be frozen at –70°C at this point for up to several weeks.
2. Add 0.2 ml PCIA and ~0.4g of acid washed beads (soaked in nitric acid, washed extensively with deionized water until neutralized, and then baked in a 200°C oven until dry).
3. Vortex in the Tomy shaker for 2.5 minutes.
4. Add 0.3 ml PCIA and 0.3 ml extraction buffer containing SDS, vortex 1 minute, and spin 15K, 5 minutes.
5. Remove aqueous phase to a new tube, and repeat extraction with an equal volume of PCIA.
6. Spin 15K, 5 minutes, and remove the aqueous phase to a new tube. Repeat extraction if the interphase is cloudy.
7. Add 2 volumes of 95% ethanol containing 0.05% diethylpyrocarbonate. Place at –70 for 1 hr. Spin at 4°C for 20 minutes, 15K.
8. Wash pellet twice with 0.5 ml cold 75% ethanol containing diethylpyrocarbonate. Dry in Speed Vac.
9. Resuspend in 100 ul DEPC-treated water.

Solutions

Extraction Buffer

0.5 M sodium chloride
0.2 M Tris pH 7.6
0.01 M EDTA
1% SDS
0.1% Diethylpyrocarbonate (DEPC)
Note: add sodium chloride, EDTA and SDS and q.v. to 80 ml, autoclave to kill DEPC, then add Tris (made with DEPC-treated water)

Extraction Buffer minus SDS

0.5 M sodium chloride
0.2 M Tris pH 7.6
0.01 M EDTA
0.1% DEPC
Note: add sodium chloride and EDTA, q.v. to 80 ml, autoclave to kill DEPC, then add Tris (made with DEPC-treated water)

PCIA

25 ml phenol
24 ml chloroform
1 ml isoamyl alcohol
50 ml extraction buffer minus SDS