RNA EXTRACTION PROTOCOL (revised 6/1/01)

REAGENTS

Guanadinium Thiocyanate	MOPS (free acid)
1.0 M Sodium Citrate pH 7.0	2.0 M Sodium Acetate pH 4.0
10% Sarcosyl	Formaldehyde
2-mercaptoethanol	Formamide
water saturated phenol pH 4.0-7.0	10% SDS
Na2EDTA	49:1 Chloroform:Isoamyl Alcohol

SOLUTIONS

SOLUTION D (100 ml)	Final Concentration	Amount
Guanadinium Thiocyanate	4.0 M	47.26 g
1.0 M Sodium Citrate	25 mM	2.5 ml
10% Sarcosyl	0.5%	5.0 ml
2-mercaptoethanol	0.1 M	720 µl
RNAse-free water		to 100 ml

filter sterilize through 0.2 µm filter

NOTE: Solution D may be made ahead of time and stored at room temp for one month, but the 2-mercaptoethanol must be added immediately before use.

PROTOCOL

1) Aspirate off media
2) Lyse cells in Solution D
   T-150 = 4 ml Solution D
   Transfer to pre-chilled 30 ml Oakridge centrifuge tube; make sure there is enough space for additions below.
3) Sequentially add:
   a) 0.1 volume 2.0 M NaOAc - mix well
   b) 1 volume phenol - mix well
   c) 0.2 volume chlorofom/isoamyl alcohol
4) Vortex 10 sec
5) Incubate on ice for 15 min
6) Centrifuge 10,000 x g, 20 min, 4 °C
7) Transfer aqueous phase to a new 50 ml conical
8) Precipitate with 1 volume isopropanol, $\geq$1 hour, -20 °C
9) Centrifuge in 50 ml conical tube, 3,000 x g, 1 hour, 4 °C
   alternatively, spin in oakridge tubes 10,000 x g, 20 min, 4 °C
10) Dissolve pellet in 0.3 ml Solution D; transfer to microfuge tube
11) Precipitate with 1 volume isopropanol, $\geq$1 hour, -20 °C
12) Centrifuge 15 min, 4 °C
13) Wash pellet with 75% ethanol
14) Dry*, dissolve in H_20**, heat to 65 °C for 10 min to completely dissolve.

*Do not over dry pellet, otherwise it will be impossible to dissolve

**use approximately 150 µl H_2O per T-150 flask

Chomczynske, P. and Sacchi, N. Analytical Biochem. 1987. **162**:156-159