

RNA FROM *E. coli* CELLS

**Solutions:**

1. Low phosphate medium (for  $^{32}\text{P}$  labeled cells):

Use a rich broth such as 2 X TB. Add  $\text{MgSO}_4$  to 0.01 M then concentrated  $\text{NH}_4\text{OH}$  to 1%. Let sit at least 2 hours and filter. Add Tris-HCl to 0.01 M and adjust to pH 7.4. Add  $2 \times 10^{-4}$  M phosphate.

2. Lysis buffer:           0.3 M Na Acetate pH 5.5  
                                  0.5% SDS  
                                  0.01 M EDTA
3. Redistilled or liquefied phenol equilibrated with sodium acetate pH 5.5 before heating.

**Procedure:**

1. For  $^{32}\text{P}$  RNA, grow overnight culture in low phosphate medium.
2. Dilute 1:200 with low-phosphate medium containing 2-5 mCi  $^{32}\text{PO}_4$  and grow at  $37^\circ\text{C}$  to a concentration of about  $10^8$  cells/ml (perhaps 5 hours).
3. Chase with one-third volume fresh medium containing  $10^{-2}$  M phosphate for about 30 min.
4. Harvest cells by spinning 5 min at 5,000 RPM and resuspend the pellet in lysis buffer using 1 ml for every 10 ml culture medium.
5. Extract with hot phenol 10 min at  $67^\circ\text{C}$  using a Pasteur pipette for mixing.
6. Separate layers by centrifugation and re-extract the aqueous layer with hot phenol.
7. Add 2 vol. cold 100% ethanol to precipitate nucleic acids.
8. After collecting RNA by centrifugation, DNA may be removed by DNase treatment and/or salt washing.

