

Protocol: Ethyl Nitrosourea Modification of Ribosome

(Reference: Vlassov et al 1981 Eur. J. Biochem. 119, 51-59;
Baudin et al., and Ehresmann 1989 Biochemistry, 28, 5847-5855
RNA-Protein Interaction Protocols by Haynes).

1. Prepare the ribosome complexes (0.4 μ M) for the modification reaction in final 25 μ l volume.
2. Prepare freshly saturated ethanolic-ENU solution by slowly adding ENU powder to 100 μ l of ethanol and vortexing briefly until ENU no longer goes into solution (about 750 mM). Spin briefly and add 5 μ l of superantant solution (appears pale yellow) to the ribosome complex. **(NOTE: ENU is highly TOXIC! Prepare & use ENU in the fume hood.)**
3. Incubate at 37 $^{\circ}$ C for 1 hour.
4. Stop the reaction by adding 25 μ l of 0.3 M Na-acetate pH 6.0 and 300 μ l of 100% ethanol.
5. Place in dry-ice/ethanol bath for 10 min.
6. Centrifuge at 4 $^{\circ}$ C for 25 min.
7. Resuspend pellets in:
 - 170 μ l of water
 - 20 μ l of 3M Na-acetate pH 5.2
 - 5 μ l of 0.5M EDTA
 - 5 μ l of 20% SDS

TOTAL = 200 μ l
8. Extract 3 times with water-saturated phenol.
9. Extract 4 times with chloroform.
10. Add 600 μ l of 100% ethanol. Place in dry-ice/ethanol bath for 10 min.
11. Centrifuge at 4 $^{\circ}$ C for 25 min.
12. Wash pellets with 100 μ l of 70% ethanol.
13. Dry pellets for 5 min in speed-vac.
14. Resuspend pellets in 50 μ l of 100 mM Tris-Cl pH 9.0.
15. Incubate at 50 $^{\circ}$ C for 15 min.

16. Add 5 μ l of 3M Na-acetate pH 5.2 and 150 μ l of 100 % ethanol.
17. Place in dry-ice/ethanol bath for 10 min. Centrifuge at 4 °C for 25 min.
18. Wash pellets with 100 μ l of 70% ethanol.
19. Dry pellets for 5 min in speed-vac.
20. Resuspend pellets in 50 μ l water. Store at -80 °C.