

### **Protocol: Preparative Scale N-Acetylation of Aminoacylated tRNA**

1. Perform standard aminoacylation of tRNA 3000-5000 pm. Add 1/10 volume of 3M sodium acetate pH. 5.0. Extract twice with acid-phenol followed by two chloroform extractions\*.
2. Ethanol precipitate with 3 volumes of ice-cold 100% ethanol. -80°C for 30 min. Spin at max speed at 4°C for 30 min. Resuspend in 200 ul of 200mM sodium acetate pH 5.0.
3. Add 2.5 ul of 99% acetic anhydride. Leave on ice for 60 minutes.
4. Add another 2.5 ul of 99% acetic anhydride. Leave on ice for 60 minutes.
5. Add another 2.5 ul of 99% acetic anhydride. Leave on ice for 60 minutes. (three additions total)
6. Ethanol precipitate with 3 volumes of ice-cold 100% ethanol. -80°C for 30 min. Spin at max for 30 min. Resuspend in 95 ul of COLD 10 mM sodium acetate pH 5.0 or 95 ul or Buffer A for HPLC purification.

\* N-Acetylation reaction can be performed without phenol and chloroform extractions (step1) and Ethanol precipitation (step 2) however it may reduce yield of final N-Acetylated product. Remember to add an appropriate (scaled up) amount of 99% Acetic Anhydride.

### **Analysis:**

- 1 If radio-labeled amino acids are used the N-Ac-tRNA can be deacylated by treatment with NaOH. Products can be separated by TLC.
- 2 Alternatively, acid-urea gel can be used to visualize products.

1. Haenni, A.L. and F. Chapeville, *The Behaviour of Acetylphenylalanyl soluble ribonucleic acid in polyphenylalanine synthesis*. Biochimica et Biophysica Acta, 1966. **114**: p. 135-148.
2. Rappoport S. and L. Y., *The chemical preparation of acetylaminoacyl-tRNA*. Methods Enzymol, 1974. **29**(0): p. 685-688.
3. Robertson, J.M. and W. Wintermeyer, *Effect of translocation on topology and conformation of anticodon and D loops of tRNAPhe*. J Mol Biol, 1981. **151**(1): p. 57-79.