

Protocol: Preparative Scale Formylation & Aminoacylation of Initiator tRNA_f^{Met}

I. Preparation of N¹⁰-formyltetrahydrofolic acid

(Reference: Cayama et al NAR 2000, 28, #12, e64)

1. Dissolve 12.5 mg of Folinic acid in 1 ml of 50 mM 2-mercaptoethanol
2. Add 110 ul of 1M HCl (May crash out, then heat to 50 C for 2 min)
3. Incubate at room temp for 3 hours (Absorbance at 355 nm increase to max). Product is N⁵, N¹⁰-methenyltetrahydrofolic acid.
4. Make 100 ul aliquotes and store at -80 C.
5. Before use: Add 1/10 vol (10 ul) of 1M Tris-HCl, pH 8.0 and 1/10 vol (10 ul) of 1M KOH. Quantitative conversion to N¹⁰-fromyltetrahydrofolic acid is indicated by decoloration. Check that the pH is close to 8.0. Use for formylation in aminoacylation reaction of initiator tRNA_f^{Met}. **Discard unused material as it is not stable at pH 8.**

II. Formylation & Aminoacylation of Initiator tRNA_f^{Met}

1. Perform standard aminoacylation/formylation reaction of 5000 pmoles of tRNA_f^{Met} (200 pm for analytical scale reaction). Make sure to add 0.3 mM final conc. of N¹⁰-fromyltetrahydrofolic acid in the aminoacylation reaction and a source for formyltransferase enzyme (either S100 extract or purified methionyl-tRNA formyltransferase).
2. Add 1/10 vol of 3M sodium acetate pH 5.0. Extract twice with acid-phenol followed by two chloroform extractions.
3. Ethanol precipitate with 3 volumes of ice-cold 100% ethanol. -80 C for 30 min. Spin at 4 C for 30 min. Resuspend pellet in 50-100 uL of 300 mM sodium acetate pH 5.0
4. Purify using a G-25 spin column pre-equilibrated in 300 mM sodium acetate pH 5.0.
5. Ethanol precipitate with 3 volumes of ice-cold 100% ethanol. -80 C for 30 min. Spin at 4 C for 30 min. Resuspend pellet in 50-100 uL of 10 mM sodium acetate pH 5.0
6. Store aliquotes in -80 C.

A. TLC Analysis for Formylation Level using [³⁵S-Met]:

1. Add 1 ul of fMet[³⁵S]-tRNA_f^{Met} to 20 ul of (1:10 diluted) Ammonium hydroxide.
2. Incubate at 55 C for 20 min to deacylate the tRNA.
3. Dry down the reaction mixture in speed vac.

4. Resuspend in 2 ul of 10 mM 2-mercaptoethanol.
5. Spot on TLC plate (Silica gel-60, 5 x 10 cm, EM Science).
6. Develop TLC in 4:1:1 :: 1-Butanol: Acetic acid: Water, for about 90 min.
7. Dry TLC either under heat lamp or using hair dryer. Wrap it in saran-wrap. Expose to PhosphorImager screen overnight. Quantitate level of formylation.

B. Acid-Urea Gel

Alternatively, acid-urea gel can be used to determine the level of aminoacylation if the amino acid is not radioactive. Acid-urea gel does not resolve the formylated tRNA very well from the aminoacylated tRNA.

Reaction Scheme:

5-Formyltetrahydrofolate/Leucovorin/Folinic acid (Sigma: Calcium salt, #F7878) ----->
5, 10-methenyltetrahydrofolate-----> 10-formyltetrahydrofolate