Determining the efficiency of aminoacylation reaction

1. Properly fold the tRNA in 30 mM HEPES (pH 7.0) and 30 mM KCl by heating to 70 °C for 5 min after which 15 mM $MgCl_2$ is added and the reaction is then allowed to cool to room temperature over a period of 30 min by transferring to metal tray containing hot water.

2. Perform small scale aminoacylation reaction with [^{35}S] methionine or [^{14}C] phenylalanine. Example for *E. coli* $tRNA^{fmet}$ is described below:

30 mM Hepes (pH 7.0)
30 mM KCl
15 mM $MgCl_2$
8 mM ATP (pH 7.0)
10 mM DTT
20 µM Methionine
0.05 µM [^{35}S] methionine (specific activity = 5,000 cpm/picomoles of methionine)
5 µl of MetRS or S-100 extract
10 µM *E. coli* $tRNA^{fmet}$
Final volume = 100 µl (make enough for duplicates and control reactions)

3. Incubate at 37 °C for 20 min.
4. For calculating specific activity of the reaction mix: Directly spot 2 µl of the reaction mix from step 2 onto filter and count without washing.
5. Take 50 µl aliquot of the aminoacylation reaction and mix with 50 µl of carrier (1 µg/ml BSA, 1M sodium acetate pH 5.2) and 1 ml of precipitation mix (2.5% trichloroacetic acid in 50% ethanol).
6. Place on ice for 20 minutes.
7. Filter the above mix through Whatman GF/C filters using the Millipore Filtration setup.
8. Wash the filter with 5 ml of 5% trichloroacetic acid. REPEAT this step 4 times.
9. Wash the filter with 5 ml of 95% ethanol. REPEAT this step 4 times.
10. Dry the filter under heat lamp for 10 minutes.
11. Add 4 ml of scintillation cocktail and count in a scintillation counter for the correct isotope.

Controls:

Perform reaction without any tRNA for calculating background.