10-Formyl-THF Synthetase Assay

Assay monitors the ATP-dependent formylation of THF to 10-formyl-THF. 10-formyl-THF is converted non-enzymatically to by 5,10-methenyl-THF the addition of acid to stop the reaction. The absorbance at 350 nm is used to calculate the amount of product formed, using ε_{350} = 24900 $M^{-1}cm^{-1}$

Stocks

1 M Tris-SO_4, pH 7.5
1 M KCl
1 M NH_4formate, pH 7.5
[titrate 1 M formic acid (prepared from 88% formic acid = 23.4 M) with NH_4OH to pH 7.5]
0.1 M $MgSO_4$
50 mM K•ATP, pH 7.5
(0.138 g/5 ml; adjusted to pH 7.5 with KOH)
10 mM THF (prepared as described)

Final assay concentrations are:
25 mM Tris-SO_4/100 mM KCl/10 mM $MgSO_4$/100 mM NH_4formate/5 mM K•ATP/2 mM THF

Procedure:

Prepare assay cocktail (can be stored at 4°C for up to 1 month):

1.25 ml Tris-SO_4
5 ml $MgSO_4$
5 ml KCl
5 ml NH_4formate
5 ml ATP
13.75 ml H_2O
35 ml = 100 assays at 350 µl/tube

Add 350 µl cocktail + 100 µl 10 mM THF to each tube and preincubate at 37°C for 1 min.

Initiate reaction with 50 µl enzyme/buffer = 0.5 ml total reaction volume. Incubate 10 min at 37°C.

Stop reactions with 1 ml 0.36 N HCl. Vortex, let stand at RT 5 min.

Read absorbance at 350 nm. Include a no enzyme blank. Read blank against water first – if THF is good, should read < 0.05. Re-blank spec against this, and read samples.

Data reduction:

$$A_{350} \times \frac{1uM}{.0249\ A350} \times \frac{1}{10\min} = \frac{1\ umol}{l \bullet \min} = \frac{nmol}{ml \bullet \min} \times 1.5\ ml = nmol\ product/\min = mU$$

A_{350} X 6.02 = nmol/min = mU