Methylenetetrahydrofolate dehydrogenase assay – microplate format

Stock solutions

2X buffer: 166 mM K•Hepes (pH 8.0)/334 mM KCl

20 mM NAD(P) (store at –20C)

Cocktail: mix 1 vol 2X buffer with 1 vol 20 mM NAD (prepare fresh)

CH_2-THF substrate: add 6 µl 1:10 (v/v) dilution of formaldehyde to 500 µl 10 mM THF (contains 500 mM 2-mercaptoethanol). Incubate at 37C, 5 min. Dilute 5-fold with 2.0 ml water. Store on ice. This yields a 1 mM CH_2-THF stock in 100 mM 2-mercaptoethanol.

Final volume in each well is 100 µl. Final concentrations of components are:

50 mM K•Hepes (pH 8.0)
100 mM KCl
6 mM NAD(P)
20 mM 2-mercaptoethanol
200 µM CH_2-THF

Assay

1. Add 60 µl **cocktail** to each well of 96 well microplate; pre-read blank the plate
2. Add 20 µl **enzyme** (~ 1 µg/ml for purified enzyme)
3. Intiate reactions with 20 µl **CH_2-THF substrate**. Incubate at 25-30C, 5 min.
4. Stop reactions with 200 µl 0.36 N HCl. Let stand 5 min. Read plate at 350 nm.
5. Blanks: identical wells, but add acid before enzyme.
6. Subtract blanks and calculate nmol product from A_{350}:

ε for acidified CH^+THF @ 350 nm = 24,900 $M^{-1}cm^{-1}$ = 24.9 $mM^{-1}cm^{-1}$

$$\frac{1mM}{24.9A350} \times A350 = \frac{1nmol}{\mu l} \times 300\mu l = nmol\ product$$

A_{350} X 12.05 = nmol product