Methylenetetrahydrofolate dehydrogenase assay – microplate format

Stock solutions

2X buffer:  166 mM K • Heps (pH 8.0)/334 mM KCl
20 mM NAD(P) (store at −20 C)

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

CH₂-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 CH₂-THF stock in 100 mM 2-mercaptoethanol.

Final volume in each well is 100 µl. Final concentrations of components are:
- 50 mM K • Heps (pH 8.0)
- 100 mM KCl
- 6 mM NAD(P)
- 20 mM 2-mercaptoethanol
- 200 µM CH₂-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)
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 A₃₅₀:

   ε for acidified CH⁺THF @ 350 nm = 24,900 M⁻¹cm⁻¹ = 24.9 mM⁻¹cm⁻¹

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   \frac{lnM}{24.9A_{350}} \times A_{350} = \frac{lnmol}{\mu l} \times 300\mu l = nmol \text{ product}
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   A_{350} \times 12.05 = \text{nmol product}
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