

An assay procedure for *Neurospora* malate dehydrogenase

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Abstract

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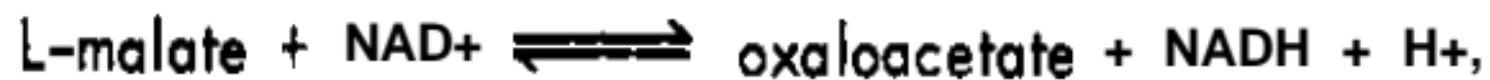
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Munkres, K. D. An assay procedure for *Neurospora* malate dehydrogenase.

Neurospora malate dehydrogenase (MDH, L-malate: NAD oxidoreductase, E.C. No. 1.1.1.37) is conveniently assayed in the reverse reaction:



by continuous spectrophotometric recording of the oxidation of NADH at 340 m μ . (Munkres and Richards 1965 Arch. Biochem. Biophys. 109: 457).

Stock solutions: (A) potassium phosphate buffer, 0.111 M, pH 7.4. Equilibrate at 25°C; (b) oxaloacetate (M.W. 132), 0.012 M, pH 7.4. Dissolve 8 mg of oxaloacetic acid in 5 ml cold phosphate buffer A. Store at 4°C. Discard after 5 days. (C) NADH (NADH.2H₂O, F.W. 696, Sigma Grade 111, 98%). Dissolve 7.10 mg in 5 ml cold distilled water (2×10^{-3} M). Store at 4°C. Discard after 5 days. (D) sodium phosphate, 0.05 M, pH 7.0. Store at 4°C.

Assay procedure: To 0.85 ml of A in a microcuvette of 1 cm lightpath, add 0.05 ml each of B and C and equilibrate in a thermoregulated (25°C) sample chamber of a spectrophotometer for at least one minute. A matched cuvette containing water is placed in the reference chamber. The absorbancy at 340 m μ should be about 0.650. Enzyme (diluted at least 5 min prior to assay in buffer D) is added (0.05 ml) to the sample cuvette, the cuvette is inverted with a Parafilm cover, and the change in

absorbance at 340 m μ is recorded for at least 3 min. An appropriate final dilution of enzyme from mycelial extracts containing about 5 mg protein is generally 2.5×10^{-4} . One unit of enzyme is defined as that amount of protein catalyzing the oxidation of one μ mole of NADH in the first minute of reaction. The initial reaction velocity is linear up to 5 min and proportional to protein concentration when the measured activity is less than 1/80 of a unit (corresponding to an absorbance change of less than 0.08 per minute). Specific activity is expressed as units per mg of protein. - - - Department of Biologic.1 Sciences, Stanford University, Stanford, California.