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:

L-malate + NAD^+ $\rightleftharpoons$ oxaloacetate + NADH + H^+,

by continuous spectrophotometric recording of the oxidation of NADH at 340 mμ. (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 ($NADH.2H_2O$, 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 mμ 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

absorbancy at 340 mμ 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 $\times 10^{-4}$. One unit of enzyme is defined as that amount of protein catalyzing the oxidation of one μmole 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 meowed activity is less than 1/80 of a unit (corresponding to an absorbancy 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.