3,3',5,5'-Tetramethylbenzidine

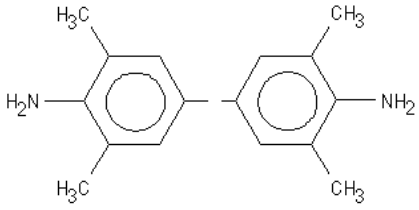
TMB
Product code A3840

Description

Formula: $C_{16}H_{20}N_2$
Molecular weight: 240.35 g/mol
CAS-No.: 54827-17-7
HS-No.: 29215990
EC-No.: 2593646
Melting point: 166 - 170°C
Solubility (water): insoluble
Solubility (organic solvents): soluble (DMSO, 96 % Ethanol, Acetone, Chloroform, Toluene)
Storage: room temperature
Disposal: 3

Specification:
Assay .....min 98 %
Loss on drying ..... max. 0.5 %
Residue on ignition ..... max. 0.2 %

TMB is oxidized during the enzymatic degradation of H_2O_2 by horse radish peroxidase. The structure of TMB can be seen in Figure 1. The oxidized product of TMB has a deep blue color. A clear yellow color is formed after addition of the acidic stop solution (0.1 M H_2SO_4). For detection of oxidized TMB, determine the OD of the yellow color in a standard ELISA plate reader at 450 nm when the reaction has been stopped with acid. If the reaction is not stopped with acid, the blue color can be measured at 655 nm.



The structure of 3,3',5,5'-tetramethylbenzidine (TMB).

TMB is an aromatic amine that undergoes oxidation by the higher oxidation states of heme peroxidases (compounds I and II) thereby serving as a reducing co-substrate. One electron oxidation of TMB results in a radical cation that forms a charge transfer complex with the unoxidized compound. This charge transfer complex absorbs at 652 nm (ε = 39,000) [1]. The completely oxidized form (diimine) absorbs at 450 nm (ε = 59,000) and is formed by two sequential one-electron oxidations of TMB [1, 2]. Thus the stoichiometry of oxidation is 0.5 mole charge transfer complex (λ_{max} = 652 nm) or 1 mole of diimine (λ_{max} =450 nm) formed (or TMB oxidized) per mole of hydroperoxide reduced by the peroxidase.

AppliCation in Peroxidase Assays

The Peroxidase Substrate solution includes TMB (0.416 µM) and hydrogen peroxide (0.832 µM). Depending on the assay system, incubation times of 5 minutes to 1 hour in the dark at room temperature have been descirbed. Check the reaction for a color change. The wells should turn blue. To stop the reaction add 100 µl 0.1 M H_2SO_4. (For the preparation of 1 liter of 0.1 M H_2SO_4 (1000 ml), mix: 50 ml 2 M H_2SO_4 with 950 ml distilled water.

Stock solution: 3,3',5,5'-Tetramethylbenzidine 10 mg/ml (41.6 mM) in DMSO.

Working solution concentration: 1 : 100 dilution of the stock solution in e.g. Citrate/Acetate Buffer. To prepare Citrate/Acetate Buffer titrate 0.1 M Sodium Acetate with 0.1 M Citric Acid to a final pH of 6.0.Citrate/Acetate Buffer, pH 6.0 can be stored frozen at -20°C.

Hydrogen peroxide (H_2O_2): Add 2 - 4 µl of fresh H_2O_2-solution (30%) to 10 ml of working solution.

References:

(1) Josephy, P.D., Eling, T., Mason, R.P. The horseradish peroxidasecatalyzed oxidation of 3,5,3',5'-tetramethylbenzidine. Free radical and charge-transfer complex intermediates. J Biol Chem 257, 3669-3675 (1982).

(2) Marquez, L.A., Dunford, H.B. Mechanism of the oxidation of 3,5,3',5'-tetramethylbenzidine by myeloperxidase determined by transient-and steady-state kinetics. Biochemistry 36, 9349-9355 (1997).

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