TMB, ULTRASENSITIVE

A Single Component-Soluble Substrate for Kinetic and Endpoint Assays of Horseradish Peroxidase

ALTERNATE NAME: 3,3',5,5'-Tetramethylbenzidine; TMBUS

CATALOG #: 1215-100

AMOUNT: 100 ml

STORAGE CONDITIONS: 2-8°C

SHELF LIFE: Stable for up to 12 months at -20°C

TMBUS SOLUTION: Contains TMB, 2.08 mMol L^{-1} and Hydrogen Peroxide, citric acid buffer at pH 3.3. Also contains non-toxic proprietary stabilizers. *Warm to assay temperature before use.*

INTRODUCTION:

3,3'5,5'-Tetramethylbenzidine (TMB) has been shown to be a safe-sensitive substrate for the assay of horseradish peroxidase (HRP). Initially, in the presence of HRP and hydrogen peroxide, a one-electron oxidation product is formed. This compound, a cation free radical, is blue in color with an adsorption maximum at 653 nm. Further reaction with HRP/H_2O_2 or acidification of the radical with acid yields the diimine terminal oxidation product adsorbing light at 450 nm. The extinction coefficient of the radical ($E_{653\ nm}$ = 3.9 x 10^4 mol^{-1} cm^{-1}) and diimine ($E_{450\ nm}$ = 5.9 x 10^4 mol^{-1} cm^{-1}) provide a remarkably sensitive system for the assay of HRP and HRP labeled probes. TMBUS, available from Biovision Inc., is a single component reagent stable at room temperature and not sensitive to normal laboratory light. It is optimized with respect to TMB and hydrogen peroxide concentrations and yields a linear response with the concentrations of HRP usually employed in immunologic assays.

ASSAY DESCRIPTION:

After completion of analyte binding to a solid phase and reaction with a HRP labeled probe, TMBUS solution is added. Oxidation of TMB produces a blue reaction product that is measured at 650 nm. The color formation as a function of time can be recorded or the reaction stopped with sulphuric acid after a fixed interval. Increased sensitivity can be achieved by converting the blue radical to the diimine by addition of acid. The resulting yellow chromogen is measured immediately at 450 nm.

STOP SOLUTION:

0.3 Mol/L Sulphuric acid for stopping reaction and preserving blue chromogen. *(Not provided).*

NOTE: Reagent grade water must contain less than 10^{-7} Mol L^{-1} of iron or copper salts otherwise unreacted TMB will be converted non-enzymatically to the diimine.

FOR RESEARCH USE ONLY! Not to be used in humans.

PROTOCOL:

1. Complete all required incubations with antibodies, probes and HRP labeled reagents.
2. Wash plate wells at least 4 times with phosphate buffered saline or tris buffered saline containing 0.1% Tween-20.
3. After the final wash, shake and blot all residual buffers from plate wells.
4. Add 0.1 ml of TMBUS Solution to appropriate wells and incubate 5-30 minutes.

   NOTE: The reaction time will depend upon the activity of the HRP probe. If color develops too briskly, zero order kinetics will not prevail. Dilution of a probe, antibody, HRP labeled reagent may be required.
5. The reaction can be monitored as a function of time for kinetic assays or stopped with 0.1 ml of 0.3 Mol/L of sulphuric acid or 0.1% sodium fluoride and read at 650 nm.
6. If the procedure demands conversion to the yellow diimine, add 0.1 ml of either acid or record the absorbance within 5 minutes.

NOTE: Protect from direct sunlight. Discard if solution is blue or turbid.

RELATED PRODUCTS:

- ABTS™ **(Cat #: 1212-100)**
- TMB, HIGH KINETICS **(Cat #: 1216-100)**
- SuperMOUNT™ Mounting Medium **(Cat#: 1211-20)**

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