(For Research Use Only. Not For Use In Diagnostic Procedures!)

FineTest®

TMB Substrate

Catalogue No.: E024

Size: 10ml/50ml/100ml/500ml

Product Feature

TMB (3,3 ',5,5 '-Tetramethylbenzidine), is a common substrate for horseradish peroxidase (HRP), suitable for enzyme-linked immunosorbent assay (ELISA). When catalyzed by horseradish peroxidase or other peroxidase enzymes, TMB produces soluble blue products. Blue products can usually be determined at 370nm or 620-650nm. Adding acid to terminate the reaction, the solution turns yellow, and the absorbance can be measured at 450nm.

TMB Substrate is a highly sensitive, single-component TMB color developing solution. Using the latest TMB color development technology, TMB and other related reagents are prepared in a single solution, which is convenient to use. Good stability. The reading is stable after the color development terminates. Low background, small difference between batches. The reagent is generally colorless, sometimes slightly blue or light yellow.

Reagent Composition

Form: Liquid.

Storage: Store at 2-8°C. Store in the Dark. Valid for 2 years.

Storage buffer pH: 3.3-4.0

Constituents: 0.01% 3,3',5,5'-Tetramethylbenzidine, 99% Water, 0.1% Hydrogen peroxide.

Directions

1, Put the whole bottle of TMB into the 37°C incubator to equilibrate for 30min.

2, After incubation of HRP conjugate, remove the cover, and then wash the plate with wash buffer five times.

3, Add 90ul TMB Substrate into each well, seal the plate and static incubate at 37°C in dark within 10-20 minutes. Run the microplate reader and preheat for 15min.
(Notes: Please do not use the reagent reservoirs used by HRP couplings. The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells. Weaker or stronger color intensity is unacceptable.)

4, The substrate reaction can be stopped using equal volumes of 1 N HCL, 0.6 N sulfuric acid, or one of the stop solutions (E026). Keep the liquid in the well after staining. Add 50ul stop solution into each well. The color will turn yellow immediately. The order for adding stop solution and TMB substrate solution is the same.

5, OD Measurement: Read the O.D. absorbance at 450nm in a microplate reader immediately and calculate.

Notes

1, If the intensity of color development needs to be reduced, it is recommended to shorten the color development time or, in immunoassay, further dilute the HRP conjugate or HRP conjugated antibody. It is not recommended to dilute the TMB color solution yourself, if you do need to, please contact us.

2, TMB should be stored away from light at 2-8 ° C to avoid exposure to light, heat, metal ions or peroxidase.

3, It should be discarded when becoming turbid or changing to a darker blue color.

4, For your safety and health, please wear a lab coat and disposable gloves during the operation.