

— PROTOCOL —

TMB ELISA Substrate

Catalog Number: 50MLx2 / Set

Storage: 4°C

Description

Bio-Helix's TMB ELISA Substrate, known as 3,3',5,5' -tetramethylbenzidine, is a chromagen that detects horseradish peroxidase (HRP) activity when oxidized with hydrogen peroxide and yields a blue color with major absorbance at 370 and 652nm. The substrate is supplied as a ready to use, mildly acidic solution. The unreacted substrate should be colorless or very light yellow in appearance. The reaction can be stopped using appropriate stop solution, either the sulfuric or phosphoric acid, and producing a soluble yellow reaction product with the absorbance at 450nm.

Highlights:

- Ready-to-use solutions
- No hydrogen peroxide required
- No filtering required
- Significantly more sensitive than leading manufacturers on the market.

Methods

1. Equilibrate the TMB ELISA Substrate to room temperature before using.
2. Mix of equal volume TMB ELISA Substrate A and B solution and then add 100 µL mixture to each microplate well.
3. Incubate in dark for 20-30 minutes or until desired color develops.
4. Add 100 µL stop solution (2N sulfuric acid) to each well.
5. Read the absorbance of each well at 450 nm within 15 minutes.