**TMB Blue**  
Highest Sensitivity Substrate-Chromogen  
Ready-to-use, Non-Flammable  

**Code S1601**  

**Intended use**  
For Laboratory Use.  

This substrate-chromogen contains 3,3’,5,5’-tetramethylbenzidine (TMB). TMB Blue is for use in peroxidase-based, colorimetric techniques that call for the highest in sensitivity detection.  

TMB Blue is a stable, non-flammable and safe alternative to other peroxidase chromogens. TMB has been reported to be non-carcinogenic.  

This substrate-chromogen is recommended for peroxidase-based enzyme immunoassays. Upon oxidation, TMB forms a water-soluble blue reaction product that can be measured spectrophotometrically at 650 nm. Upon acidification, the reaction product becomes yellow with an absorbance peak at 450 nm.  

**Reagent**  
TMB Blue substrate-chromogen is a stabilized, ready-to-use reagent that requires no further preparation.  

**TMB Chromogen**  
Stabilized peroxide substrate and 3,3’,5,5’-tetramethylbenzidine in a dilute buffer solution.  

**Precautions**  
1. For professional users.  
2. This TMB reagent is sensitive to contamination from a variety of oxidizing agents such as bacteria, dust, metals and commonly used laboratory glassware. To avoid contamination and premature expiration, avoid contacting TMB reagent with any potential source of contamination.  
3. To avoid contamination, never pipette directly from bottle. Pour out required amount into a beaker and pipette from beaker. Do not return excess TMB to primary storage container.  
4. TMB has been reported to be non-carcinogenic. However, personal protective equipment is recommended to avoid direct exposure.  
5. Use prudent laboratory practices when handling reagents. This includes avoiding unnecessary contact and using personal protective equipment such as chemical resistant gloves, eye protection and lab coat.  
6. As a general rule, persons under 18 years of age are not allowed to work with this product. Users must be carefully instructed in the proper work procedures, the dangerous properties of the product and the necessary safety instructions. Please refer to Safety Data Sheet (SDS) for additional information.  
7. Unused solution should be disposed of according to local, State and Federal regulations.  
8. Safety Data Sheet available for professional users on request.  

**Warning**  
**TMB Blue Substrate-Chromogen:** 10-30% Propylene carbonate, 1-5% N-Methyl-2-pyrrolidone  

- H319 Causes serious eye irritation.  
- H315 Causes skin irritation.  
- H335 May cause respiratory irritation.  
- P280 Wear protective gloves. Wear protective gloves. Wear eye or face protection.  
- P271 Use only outdoors or in a well-ventilated area.  
- P261 Avoid breathing vapor.  
- P264 Wash hands thorougly after handling.  
- P304 + P340 + IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Call a POISON CENTER or physician if you feel unwell.  
- P312 IF ON SKIN: Wash with plenty of soap and water. Take off contaminated clothing. Wash contaminated clothing before reuse.  
- P302 + P352 + IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.  
- P338 Continue rinsing.  
- P337 + P313 IF eye irritation persists: Get medical attention.  
- P405 Dispose of contents and container in accordance with all local, regional, national and international regulations.  

**Storage**  
Store at 2–8°C. Protect from light. May be frozen. Bring to room temperature and mix well before use.  

**Procedure**  
1. Add 100 µl of TMB Blue substrate-chromogen to wells containing peroxidase.  
2. Time reaction for approximately 10 minutes. Longer reaction times, up to 60 minutes, can be used if increased sensitivity is required. Positive wells will turn blue.
3. Stop reaction by adding an equal volume of acid stop solution* to each well. Positive wells will turn yellow. Negative wells will be clear.
4. If a blue tinge persists after one minute, mix contents of well to disperse stop solution.
5. Visually determine results. If desired, wells can be read spectrophotometrically at 450 nm. Blank spectrophotometer against an appropriate substrate well in which no reaction has occurred.

*Several acid solutions may be used to stop the TMB reaction. A recommended acid stop solution is 1 N H₂SO₄ or a mixture of HCl and H₂SO₄ (final concentration equals 1N HCl and 3N H₂SO₄).

Results
Unstopped Reaction
Positive wells will display a blue reaction product with an absorbance peak at 650 nm.

Stopped Reaction
Addition of an acid stop solution will increase absorbance readings by approximately 50%. Acidified reaction product will be yellow with an absorbance peak at 450 nm.

Limitations
The intensity of the acidified reaction product may change over time. For best results, read immediately after adding acid stop solution.

References