TMB Chromogen Solution (HRP-based ELISA)

Rev 1.03

Product code	Product name	Pack size
BL728A	TMB Chromogen Solution (HRP-based ELISA)	100 mL
BL728B	TMB Chromogen Solution (HRP-based ELISA)	500 mL

Product description

TMB (3,3′,5,5′-tetramethylbenzidine) is a commonly used chromogenic substrate for horseradish peroxidase (HRP). HRP catalyses the oxidation of TMB by H_2O_2, producing a blue product with major absorption peaks at 370 nm and 652 nm. Stopping the reaction with sulfuric or phosphoric acid changes the product to yellow, with a maximum absorption at 450 nm. Conventional TMB reagents are often supplied as separate A and B solutions (TMB and H_2O_2), which must be mixed before use and can readily form precipitates, making handling less convenient and increasing the potential for operating errors. This product combines the relevant reagents into a single solution using TMB chromogenic technology.

Product features

1. Ready to use, with no A/B premixing required. Detection sensitivity is no lower than that of an A/B solution system.

2. The single-solution format simplifies the procedure, reduces operating errors and provides more stable results.

3. Contains a stabilizer for long-term storage at 4 °C.

4. Provides higher detection sensitivity than other HRP chromogenic substrates such as ABTS and OPD. This TMB solution contains no organic solvents such as DMF or DMSO and is described in the source as safe and non-toxic.

Directions for use

1. Before use, remove the required quantity of single-component TMB solution and allow it to reach room temperature.

2. For ELISA, follow the assay protocol. After incubation with the HRP-labelled antibody, wash 3–5 times with an appropriate wash solution, for 3–5 minutes each time. Remove the wash solution and add 100 μL of TMB solution.

3. Generally incubate at room temperature, protected from light, for 10–30 minutes. Longer incubation may be used according to the experiment, until the desired colour intensity is reached; the reaction solution is blue at this stage.

4. Add 50 μL of 2 N hydrochloric acid (2 mol/L) or sulfuric acid (1 mol/L) to stop the reaction. The solution changes from blue to yellow. Measure absorbance at 450 nm.

For in vitro research use only. Not for diagnostic or therapeutic use. This product is not a medical device.
Contact: support@darwinscience.com

Troubleshooting

A Excessive background colour

1. If the background (the control without sample) is too strong, consider an appropriate blocking solution, such as a commercial blocker or commercial serum from the same species as the primary antibody (5–10%). Use an appropriately affinity-adsorbed secondary antibody to reduce nonspecific cross-reactivity.

2. Consider shortening colour development or reducing the secondary-antibody concentration. Use an appropriate wash-solution strength or extend the washing time.

3. The TMB substrate reaction may be too strong. To avoid precipitation, read immediately after stopping the reaction, or further dilute the primary antibody and HRP conjugate.

B No colour or weak colour

1. Increase the primary- or secondary-antibody concentration as appropriate. To check the secondary antibody, place a drop of diluted secondary antibody in a microcentrifuge tube and test whether it produces the expected colour response.

2. Use a more sensitive amplification system, such as a biotin-based detection system.

3. Extend the colour-development time as appropriate.

4. If these changes do not provide the expected result, consider a more effective primary antibody or ELISA.

Precautions

1. TMB is irritating to humans. Handle carefully and use appropriate protection to avoid direct contact or inhalation.

2. For scientific research by professional personnel only. Do not use for clinical diagnosis or treatment, or in food or pharmaceuticals.

3. Wear a laboratory coat and disposable gloves during use.

Storage conditions

Store at 4 °C, protected from light. Shelf life: one year.

Shipping

Shipping: blue ice.