DNAbiotech Co.

INSTRUCTIONS

TMB substrate kit

Cat#: DB9510

For the rapid, sensitive and accurate measurement of protein in various samples

This product is for research use only and is not intended for diagnostic use.

Kit Components:

Item	Quantity
Reagent A	10 mL
Reagent B	1 mL
Reagent C	100 mL

Storage upon receipt:

- 2-8 ° C (Kit is shipped at ambient temperature)
- Protect from light

Note: Reagent A could be aliquoted and store at -20° C for 2 years.

Overview:

The **DNAbiotech** TMB Substrate Kit contains soluble TMB (3,3´,5,5´-tetramethylbenzidine) that is ideal for sensitive ELISA-based applications. TMB is a chromogenic substrate for horseradish peroxidase (HRP) in ELISA assay. Once oxidized by the enzyme, this substrate yields a blue product that absorbs at 370nm and 652nm. Adding sulfuric or phosphoric acid to stop the reaction changes the product to yellow that absorbs at 450nm

Procedure for using TMB for ELISAs

Note: Color variations of the TMB Solutions are normal and will not interfere with ELISA results. However, in this product as the reagents are separated, that will not happen.

1. Immediately before use, mix 1 ml of reagent A with 9 ml of Reagent C
Invert it and add 2 ul of Reagent B (using this dilution ratio, final volume can be change)
2. Add 100µL of the TMB solution to each microplate well.
3. Incubate plate at room temperature for 15-30 minutes or until the desired color develops.

Note: High concentrations of HRP yield a greenish solution. Stop the reaction before any wells display a green product. A precipitated product indicates the presence of too much HRP and the need to optimize experimental conditions.

4. Stop reaction with 100µL of 2M sulfuric acid or 8M acetic acid with 1M sulfuric acid.
5. Measure the absorbance of each well at 450nm.

References

1. Bos, E.E., et al. (1981). 3,3'5,5'-Tetramethylbenzidine as an Ames test negative chromogen for horseradish peroxidase in enzyme immunoassay. J Immunoassay 2:187-204.
2. Josephy, P., et al. (1982). The horseradish peroxidase-catalyzed oxidation of 3, 5,3',5' Tetramethyl benzidine. J Biol Chem 257(7):3669-75