

## Product Information

### 3,3',5,5'-Tetramethylbenzidine

tablet, 1 mg substrate per tablet

Catalog Number **T5525**

Storage Temperature 2–8 °C

#### Product Description

3,3',5,5'-Tetramethylbenzidine (TMB) is a chromogenic substrate suitable for use in ELISA procedures, which utilize horseradish peroxidase conjugates.<sup>1-5</sup> This substrate produces a soluble end product that is blue in color and can be read spectrophotometrically at 370 or 655 nm. The reaction may be stopped with 2 M H<sub>2</sub>SO<sub>4</sub>, resulting in a yellow solution that is read at 450 nm.

Each tablet contains 1 mg of TMB substrate. The product is available in packages of 50 or 100 tablets. Custom packaging and bulk purchase information are available upon request.

#### Precautions and Disclaimer

This product is for Research Use Only. Not for Use in Diagnostic Procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

#### Preparation Instructions

##### 0.05 M Phosphate-Citrate Buffer

- Dissolve one phosphate-citrate buffer tablet (e.g., Catalog Number P4809) in 100 mL of ultrapure water with stirring, to yield a 0.05 M phosphate-citrate buffer, pH 5.0.  
**Or**
- Add 25.7 mL of 0.2 M dibasic sodium phosphate (e.g., Catalog Number S0876), 24.3 mL of 0.1 M citric acid (e.g., Catalog Number C7129) and 50 mL of ultrapure water. Adjust the pH to 5.0, if necessary.

#### TMB Substrate Solution

- Dissolve one TMB tablet in 1 mL of DMSO, and add to 9 mL of 0.05 M Phosphate-Citrate Buffer, pH 5.0. Add 2 µL of fresh 30% hydrogen peroxide (e.g. Catalog Number H1009) per 10 mL of substrate buffer solution, immediately prior to use.  
**Or**
- Dissolve one TMB tablet in 1 mL of DMSO, and add to 9 mL of 0.05 M phosphate-citrate buffer, pH 5.0, containing 0.03% sodium perborate (capsules, Catalog Number P4922).

#### Stop Solution

The reaction may be stopped by the addition of 50 µL of 2 M H<sub>2</sub>SO<sub>4</sub> per 200 µL of reaction mixture.

#### Storage/Stability

Store the TMB tablets at 2–8 °C. Protect from heat, light, and moisture. Allow tablets to reach room temperature prior to use.

#### References

1. Bos, E. *et al.*, *J. Immunoassay*, **2(3-4)**, 187-204 (1981).
2. Wróblewska, B. *et al.*, *Int. J. Food Sci. Tech*, **39(8)**, 839-850 (2004).
3. Doig, N.M. *et al.* *J. Neurosci.*, **30(44)**, 14610-14618 (2010).
4. Szymkiewicz, A., and Chudzik-Kozłowska, J., *Acta Alimentaria*, **43(2)**, 193-291 (2014).
5. Rosenqvist, E. *et al.*, "Determination of Antibody Responses to Meningococcal Antigens by ELISA", in *Meningococcal Vaccines: Methods and Protocols* (A.J. Pollard and M.C.J. Maiden, eds.). *Methods in Molecular Medicine*, Springer/Humana Press, pp. 255-273 (2001).
6. Evans, D. *et al.*, *Sci. Rep.*, **7**, 685 (2017).
7. Tan, P. *et al.*, *Mol. Cell*, **68(2)**, 293-307 (2017).

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