

## Product Information

### 3,3',5,5'-Tetramethylbenzidine Liquid Substrate, Super Slow, for ELISA peroxidase substrate

Catalog Number **T5569**

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 (HRP) conjugates.<sup>1-4</sup> This substrate produces a soluble end-product that is blue in color and can be read spectrophotometrically at 370 or 650 nm. The reaction may be stopped with acid, resulting in a yellow solution that is read at 450 nm.

This product is supplied as a ready-to-use, one-component peroxidase substrate containing TMB in a mildly acidic buffer. Rate kinetics are ~60% slower than traditional TMB formulations. Prior to the reaction with HRP, the substrate should be a colorless to light bluish-green solution. The substrate system develops a blue reaction product when reacted with HRP in microwell applications. For end-point assays, acid can be used to stop the reaction, yielding a yellow end-product. Since this substrate produces a soluble reaction product, it is not recommended for histochemistry or blotting.

Several publications have cited use of this product in their protocols.<sup>5-8</sup>

#### Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

#### Storage/Stability

Store at 2–8 °C. This substrate is light sensitive and should be protected from direct sunlight or UV sources.

#### Procedure

- Bring to room temperature before use.
- Following the reaction with HRP, a blue reaction product forms that may be read at 370 nm or between 620 and 655 nm. For end-point assays, the reaction can be stopped by the addition of a volume of 1 M or 2 M HCl, or 0.5 M H<sub>2</sub>SO<sub>4</sub>, equal to the volume of the substrate reaction in the well. The resulting yellow end-product, which is stable for at least one hour, can then be read at 450 nm. A preformulated Stop Reagent, Catalog Number S5814, is available for this application at 450 nm.
- End-point assays can also be read at 650 nm using another Stop Reagent, Catalog Number S5689.
- To reduce the intensity of a reaction, it is suggested that the antibodies or conjugates be diluted.

#### 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. Lissilaa, R. *et al.*, *J. Immunol.*, **185(9)**, 5512-5521 (2010).
6. Bonvin, P. *et al.*, *Methods Enzymol.*, **570**, 73-85 (2016).
7. Liu, C.-C. *et al.*, *Neuron*, **96(5)**, 1024-1032 e3 (2017).
8. Wildburger, N.C. *et al.*, *Sci. Rep.*, **7(1)**, 9520 (2017).

AGW,GCY,MAM 02/19-1