

**Product Information** 

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

≥98% (TLC)

#### T2885

# **Product Description**

CAS Registry Number: 54827-17-7

Molecular Formula:  $C_{16}H_{20}N_2$ Formula Weight: 240.34

Structure:

$$H_3C$$
 $H_2N$ 
 $H_3C$ 
 $CH_3$ 
 $CH_3$ 
 $CH_3$ 

3,3',5,5'-Tetramethylbenzidine (TMB) is a colorimetric substrate that is used with peroxidase (HRP) and peroxidase-coupled systems. The reaction of TMB with peroxidase produces a soluble end product, which is blue in color and can be read spectrophotometrically at 370 nm or 655 nm. The reaction can be stopped with 2 M sulfuric acid and read at 450 nm. This results in a 2-fold to 4-fold enhancement of the sensitivity of the reaction.

This T2885 product is the free base form of TMB. The hydrochloride salt of TMB can be directly dissolved in aqueous media. For this reason, the hydrochloride salt is the frequently used form of TMB. However, both the free base and the hydrochloride salt forms of TMB will give identical results.

TMB has been used in the quantitative determination of hemoglobin and cytochemical staining for peroxidase.<sup>3</sup> Several publications,<sup>4-5</sup> theses<sup>6-7</sup> and dissertations<sup>8-13</sup> have cited use of T2885 in their research protocols.

## 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 the product at 2-8 °C. It remains active for a minimum of two years as supplied.

## **Preparation Instructions**

A TMB stock solution can be prepared by dissolving 1 mg/mL in DMSO. The working solution is made by diluting 1 mL of the DMSO stock with 9 mL phosphate-citrate buffer, pH 5.0, and then adding 2  $\mu$ L of fresh 30% hydrogen peroxide (Cat. No. H1009) per 10 mL of substrate/buffer solution immediately prior to use.

Stock solutions of TMB in DMSO can be frozen in 1-2 mL aliquots at -20 °C. Solutions appear to remain active for at least two years when stored in this manner. Any oxidation would be revealed as a color change in the working solution.

## References

1

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