

**Product Information** 

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

Tablet, 1 mg substrate per tablet

#### T5525

# **Product Description**

Storage Temperature: 2-8 °C

Structure (TMB):

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

3,3',5,5'-Tetramethylbenzidine (TMB) is a colorimetric substrate that is used with horseradish peroxidase (HRP) and peroxidase-coupled systems.<sup>1</sup> The reaction of TMB with peroxidase produces a soluble end product,<sup>2</sup> 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 (H<sub>2</sub>SO<sub>4</sub>) and read at 450 nm.

Each T5525 tablet contains 1 mg of TMB substrate. T5525 is available in packages of 50 tablets or 100 tablets per box, individually foil wrapped for ease of use, storage, and safety.

Several theses<sup>6-8</sup> and dissertations<sup>9-18</sup> have cited use of T5525 in their research protocols.

# 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.

# Preparation Instructions

# TMB Substrate Solution

# Option 1

- Dissolve one TMB tablet in 1 mL of DMSO.
- Add dissolved TMB solution to 9 mL of 0.05 M Phosphate-Citrate Buffer, pH 5.0.
- Add 2 µL of fresh 30% hydrogen peroxide per 10 mL of substrate buffer solution, immediately prior to use.

#### Option 2

- Dissolve one TMB tablet in 1 mL of DMSO.
- Add dissolved TMB solution to 9 mL of 0.05 M phosphate-citrate buffer, pH 5.0, containing 0.03% sodium perborate (capsules, Cat. No. P4922).

## Phosphate-Citrate Buffer Preparation

To prepare 0.05 M phosphate-citrate buffer, pH 5.0:

## Option 1

 Dissolve one phosphate-citrate buffer tablet (such as Cat. No. P4809) in 100 mL of ultrapure water with stirring.

#### Option 2

- Mix 25.7 mL of 0.2 M dibasic sodium phosphate, 24.3 mL of 0.1 M citric acid, and 50 mL of ultrapure water.
- Adjust the pH to 5.0, if necessary.

#### **Stop Solution**

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

# Precautions and Disclaimer

This product is 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.

#### References

1

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