ImmunoBioScience Corp. (IBSC) *DATA SHEET*

Tetramethylbenizidine (TMB) For ELISA (Ready-to-use) Peroxidase chromogen substrate (One component)

**Catalog number:** AR-8221-01 50 ml

AR-8221-02 100 ml

AR-8221-04 500 ml

**Description**: 3,3'5,5’- tetramethylbenzidine (TMB) is a soluble chromogen substrate for horseradish peroxidase detection systems for ELISA. This is ready to use one component system and gives soluble blue color with peroxidase which absorbs at 653 nm; on acidification gives stable yellow color that absorbs at 450 nm. The extinction coefficient of blue color ( E653 nm = 3.9 x 104 mol-1 cm-1 ), and the yellow color is ( E450 nm = 5.9 x 104 mol-1 cm-1 ). TMB yields stable blue color as end product ., that can be measured at 650 nm; the color formation is function of time The reaction is stopped by adding acid solution it yields a yellow color that can be measured at 450 nm. This yellow color is 1.5 X more sensitive than blue color. This TMB solution contains TMB, 2.08 mMol L-1, Hydrogen Peroxide substrate, citrate buffer and non-toxic proprietary stabilizers.

**Intended Use**: ELISA This TMB solution is ready to use and does not require any preparation.

**Reagent required but not supplied**: 0.5M sulfuric acid. (Stop reagent)

**Storage: 2-8°C**

**Application:**

1. After the peroxidase reaction, wash ELISA plate with buffer 5-7 X (this buffer should not contain any sodium azide because peroxidase is inactivated). Remove air bubbles thoroughly from the ELISA plate.
2. Add 50µl of ready to use TMB in each well of ELISA plate.
3. Monitor color development until the positive control has desired blue color; normally it takes 2-10 minutes.
4. Read absorbance using microtiter plate reader at 653 nm.
5. To stop the reaction, add 50µl of 0.5M sulfuric acid. Mix thoroughly and read absorbance at 450 nm immediately because the yellow color intensity may change overtime.

*The optimum time of incubation for TMB should be determined by the individual lab.*

***The reaction mixture can be discarded according to city, county, state, province or***

. ***Country’s regulations.***

DO NOT ADD THIMEROSAL IN TMB OR ANY FINAL REACTION MIXTURE; IF ADDED THE YELLOW COLOR WILL DISAPPEAR IN FEW SECONDS.

**Limitation and warranty:** Our warranty is limited to the actual price paid for the product. We are not liable for any property damage, personnel injury, time, effort or economic loss due to our product.

**MSDS:** Avoid skin and eye contact with all laboratory products. Use appropriate lab. gear, lab coat , gloves and safety glasses. Do not ingest any lab. products. This product is not approved for administration in human or animals.

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Phone: + 1 425 367 4601; Fax: + 1 425 367 4817; cell (mobile) phone: + 1 425 314 0199

Marketing phone: + 1 650 343 IBSC (4272); e-mail:anitaIBSC@aol.com

Web site: [www.immunobioscience.com](http://www.immunobiosci.com); E-mail: baderbo@gmail.com