



# Diagnopal Dynabioassay Products LTD

## ELISA Grade, Sensitive TMB-Substrate (One Component)

Product code: **DPTMB**

***\*\*This product is recommended to commercial ELISA kits and routine protocols.***

### General

TMB (3,3',5,5' tetramethylbenzidine) is a non-carcinogenic benzidine compound in a mildly acidic solution and a chromogen that yields a blue color when oxidized with hydrogen peroxide (catalyzed by HRP) with major absorbances at 370 nm and 652 nm. The color then changes to yellow (measured at 450 nm) upon addition of acid to stop the reaction. TMB is very sensitive and more quickly oxidized than other HRP substrates, resulting in faster color development.

### Form, Stability, and storage

**Diagnopal Sensitive TMB** (3,3',5,5' tetramethylbenzidine) is Liquid solution in amber reagent bottle. It should be Stored at 2 – 8°C. Do not freeze. Stable for 24 months from date of manufacturing, when stored at 2 – 8°C.

**APPEARANCE/BACKGROUND:** The expected appearance of the solution is colorless to very light yellow. background of un-reacted substrate at 650 nm is 0.01 - 0.1. Some fine floating particles may occur during storage, this will not affect Background or test results.

### RECOMMENDED HANDLING

Diagnopal TMB-Peroxidase Substrate (1- Component) is extremely sensitive to certain handling and storage conditions. However, shipment must be done in 2-8C temperature and keeping products a in RT will seriously affect the shelf life and sensitivity of it.

**\*\***Avoid exposure to sunlight, air, and extreme temperatures, however exposure to indirect laboratory lighting for 8 hours is not harmful.

**\*\*** Never pipette directly from bottle. Pour out needed amount into a plastic reservoir. Do not return excess Diagnopal TMB from reservoir to the primary container.

**\*\*** Avoid exposure to silica-based materials including borosilicate glass, metals, bacterial contamination, or oxidizing agents.

**\*\*** HDPE and LDPE bottles should be utilized as they are received directly from the manufacturer; washing of bottles is not recommended, but if you need, acid washing and several times rinsing with D.W is a must.

**\*\*** keep main bottle at 2 – 8°C and just bring daily use volume to room temperature.

### Recommended protocol:

**To start reaction:** Add 100-200 µL of Diagnopal TMB-Substrate to each well and incubate 10-20 Min, then stop the reaction with stop solution and read the OD and results in 5-10 minutes.



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TMB-Substrate color development following incubation with peroxidase labeled conjugate will occur. Over reaction shall lead to generation of precipitated particles. In these cases, you may need make dilution in your sample for quantitative tests or report: (Analyte concentration is more than highest standard in curve).

**To Stop Reaction** For optimal performance, stop reaction by adding equal volume or less of Stop Solution to the microwell plate. This will halt color development and will turn it to yellow.

**To Read Reaction After stopping,** read at a wavelength of 450 nm. Stopped reaction should be read within 5-10 minutes.