

# HRP Substrate TMB Solutions for ELISA

## Product Description

*Uptima* TMB solutions are chromogenic reagents for peroxidase, designed for ELISA techniques, manual or automatic systems. They contain 3,3',5,5'-tetramethylbenzidine (TMB), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and proprietary catalyzing and stabilizing agents. Reaction with peroxidase develops an intense blue colour that can be read directly (at 650nm), or a deep yellow colour (read at 450 nm) after stop with an acid solution. Sensitivity is greater than classic substrates like OPD and ABTS, with very low background..

<i>Name</i>	<i>Cat. Number</i>	<i>Applications</i>
<b>TMB Solution « Standard »</b> (stability 18 months)	UP664780, 200 ml UP664781, 500 ml UP664782, 1 litre	ELISAs / HRP (classic applications)
<b>TMB Solution « Check »</b> (stability 24 months)	UPS08170, 100ml UPS08171, 500ml	ELISAs / HRP (with visual or optical controle of deposits)
<b>TMB Solution « Aqueous »</b> (stability 24 months)	UPS08181, 200 ml UPS08182, 500 ml UPS08183, 1 L	ELISAs / HRP (diagnostic kits notably)

**Storage :** +4°C protected from light. Do not freeze. (κ)

## Directions for Use

### Protocol

- The ELISA assay may be performed according to your standard protocol. Wash the microplates thoroughly to remove unbound peroxidase labelled probe (antibody, lectin, nucleotide...). Insufficient washes may lead to undesired back ground. Uptima recommends 4 washes in PBST (NaCl 150mM, phosphate 20mM, Tween20® 0.1%, pH7.5).
- Add 150 µl of TMB substrate to each microplate well. Agitate slowly to homogenize. Incubate for 30 mn at room temperature, protected from light. The colour develops immediately.  
 Note : The TMB solution is ready-to-use, it is not necessary for it to reach room temperature. The volume per well and incubation duration may be adjusted depending on the detection system. If the staining is to rapid or intense, primary or secondary antibodies should be diluted, or staining time reduced. It is not recommended at all to dilute the TMB substrate.
- Immediate reading (blue colour) :** useful for kinetic studies.  
Read the optical absorbance at 630-650 nm. Recommended wave-length is 650 nm.
- Reading after reaction-stop (yellow colour) :** sensitivity is increased 2 to 3 fold.  
Add 100 µl of stop solution (UPS29590) to each well. Positive wells become yellow. Read the optical absorbance at 450 nm. It is recommended to measure the absorbance immediately. It can be read up to 1 hour after stop-solution addition, but thereafter, signal may be 10% decreased.  
 Note : Stopping the reaction may be performed with various acid solutions. H<sub>2</sub>SO<sub>4</sub> 1M allows noticeably higher signals, but the reading should be done within 15minutes; else 20-30% or more decrease of the signal and precipitate formation may be observed affecting the accuracy and sensitivity of detection.

## Other Information

Uptima TMB substrates are optimized for direct and indirect ELISA techniques. They are not suitable for Immunohistochemistry or Western Blotting.

Recommendations for use: TMB is sensitive to certain conditions of storage and operating.

1. Exposure to light : TMB substrate is light sensitive. It should be stored in amber vials of HDPE type.
2. Material : the substrate is very sensitive to metallic ions. Only high quality plastic or glass should be used.
3. Avoid the use of caps with rubber joins : this should impair the results.
4. Don't pipet the TMB directly from the bottles : fill a suitable container first with the necessary solution volume to avoid contamination.
5. Don't leave the bottle open too long , and keep it a dark room at +4°C. Avoid important or frequent variations of temperature.

NT-UP66478 : comparison of TMB sensitivities

NT-UPS0817: stop solutions

NT-UPS0817: comparaison

### Related Products :

Peroxidase labeled Secondary Antibodies  
Streptavidine labeled Peroxidase, UP39588  
Sea Block Saturating agent, UP40301  
Albumin, 30% solution, UP90013  
Stop solution, UPS29590

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