

## TMB Solutions (HRP Substrate for ELISA)

### Product Description

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

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

**Uptima** TMB solutions are chromogenic reagents for peroxidase, designed for manual or automated ELISA techniques. 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 color that can be read directly (at 650nm), or a deep yellow color (read at 450 nm) after stopping with an acid solution. Sensitivity is greater than classic substrates like OPD and ABTS, with very low background.

Our TMB substrates are available in 3 versions:

"**standard solution**" is the highly sensitive original version (the most sensitive tested reagent);

"**check solution**" includes a red dye for visual inspection, or for the automated control of the correct distribution of the solution in microplates (empty wells, volume variations);

"**aqueous solution**" that does not contain organic solvents and is thus useful for diagnostic manufacturing (no shipping regulations).

### Directions for Use

#### Protocol

- The ELISA assay may be performed according to your standard protocol. Wash the microplates thoroughly to remove any unbound peroxidase labeled probe (antibody, lectin, nucleotide...)  
Insufficient washing may lead to undesired background.  
Uptima recommends 4 washes in PBST (NaCl 150mM, phosphate 20mM, Tween20® 0.1%, pH7.5).
- Add 150 µl of Uptima ready-to-use TMB substrate to each microplate well.  
Agitate slowly to homogenize.  
Incubate for 30 mins at room temperature, protected from light.  
See notes below for additional information (reagent storage[a], preparation[b], incubation optimization [c]).
- Immediate reading (blue color)** : useful for kinetic studies.  
Read the optical absorbance at 630-650 nm. Recommended wavelength is 650 nm.
- Reading after reaction-stop (yellow color)** : 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 decreased by 10%.  
See note [d] below for additional information

Contact your local distributor

[uptima@interchim.com](mailto:uptima@interchim.com)

## Additional Information

Uptima TMB substrates are optimized for direct and indirect ELISA techniques.

They are not suitable for Immunohistochemistry or Western Blotting.

Uptima TMB solution standard was found the best amongst tested competitors, especially regarding sensitivity of detection in ELISA (ask for comparison of TMB [NT-UP66478](#)).

The reagent is very stable, at least 18 months under proper storage conditions.

Stringent manufacturing conditions ensure excellent lot-to-lot reproducibility.

### Notes:

[a] Incorrect conditions of storage and operating may affect TMB performance:

- Exposure to light: TMB substrate is light sensitive. It should be stored in amber vials, and exposure of reagent to the light during the ELISA procedure should be limited (protect during incubation).
- Avoid important or frequent variations of temperature.
- The substrate is very sensitive to metallic ions. Only high quality plastic or glass should be used. Avoid the use of vials/caps with rubber seals: this could impair the results.

[b] TMB preparation

- The TMB solution is ready-to-use. For use, it is not necessary to reach room temperature.

Do not pipette the TMB directly from the bottle, and do not leave the bottle opened for long periods: fill a clean container first with the necessary solution volume to avoid contamination, and distribute to ELISA microplate (if not used immediately, protect from direct light exposure and keep at +4°C for no more than 1 day. It is preferable to only prepare the volume required).

[c] TMB incubation

- The volume per well and incubation duration may be adjusted depending on the detection system. If the staining is too rapid or intense, primary or secondary antibodies should be diluted, or staining time reduced. Do not dilute the TMB substrate.
- Stopping the reaction may be performed with various acid solutions. We recommend using our reagent UPS29590. H<sub>2</sub>SO<sub>4</sub> 1M allows noticeably higher signals, but the reading should be completed within 15 minutes; otherwise there may be a 20-30% or more decrease of the signal and precipitate formation may be observed, affecting the accuracy and sensitivity of detection.

## Related Documents and Products:

NT-664780: TMB comparison of sensitivity, stability

Stop solution for TMB, UPS29590

See [BioSciences Innovations catalogue](#) and [e-search tool](#):

other [Uptima reagents for ELISA procedures](#)

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

**Disclaimer :** Materials from Uptima are sold **for research use only**, and are not intended for food, drug, household, or cosmetic use. Uptima is not liable for any damage resulting from handling or contact with this product.

rev.H11E-H01E

Contact your local distributor

[uptima@interchim.com](mailto:uptima@interchim.com)

interchim

Uptima, powered by  
213 Avenue J.F. Kennedy - BP 1140  
03103 Montluçon Cedex - France  
Tél. 04 70 03 88 55 - Fax 04 70 03 82 60