



TMB ready-to-use solutions (HRP Substrate for ELISA)

Products Description

<i>Description</i>	<i>Product Number</i>	<i>Applications</i>
TMB Solution « Standard » (stability 48 months)	UP664780, 200 ml UP664781, 500 ml UP664782, 1 L	ELISAs / HRP (classic applications)
TMB Solution « Friendly » (stability 48 months)	UP664700, 200 ml UP664701, 500 ml UP664702, 1 L	ELISAs / HRP (REACH compliant)
TMB Solution « Wise » (stability 24 months)	UP664770, 200 ml UP664771, 500 ml UP664772, 1 L	ELISAs / HRP (REACH compliant No hazardous labeling)
TMB Solution « Aqueous » (stability 24 months)	UPS08181, 200 ml UPS08182, 500 ml UPS08183, 1 L	ELISAs / HRP (REACH compliant)

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 (H₂O₂), 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" is the highly sensitive original version, with low background, and the longest shelf life.

"Friendly" offers the same advantages with an increase sensitivity (especially at high HRP concentrations) and is compliant with Annex XVII for REACH

"Wise" is also REACH compliant and, moreover, it displays no hazardous labeling. Shelf life offers and shorter shelf life

"Aqueous" that does not contain organic solvents and is thus REACH compliant and more environment friendly

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):**
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 30min after stop-solution addition, but thereafter, signal may be decreased by 10%.

For use of TMB check+ solution, also read at 540nm (the addition of the acid stop to the substrate produces a color change from colorless to pink). In peroxidase-negative wells (e.g., blank wells), the appearance of a pink color (measured at 540 nm) indicates that both substrate and acid stop have been added to the assay. The absence of a 540 nm signal indicates an invalid well, as either substrate or acid stop was not added to the well. In peroxidase-positive wells, the yellow color (absorbance at 450 nm) may obscure the visual color change of the indicator. However, the absorbance change at 540 nm can still be observed spectrophotometrically.

See note [d] below for additional information

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).

FT-664780

[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.

H₂SO₄ 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-66478c](#): TMB comparison of sensitivity, stability

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

*other [Uptima reagents for ELISA procedures](#) using TMB solutions:

Stop solution for TMB, [UPS29590](#) ([Specifications Sheet](#))

Peroxidase labeled Secondary Antibodies

Streptavidin labeled Peroxidase [UP39588](#)

PBS buffer, powder pack [UP68723A](#) and TBS buffer, powder pack [UP74004A](#)

PBS and TBS blends: with non fat milk [GS4160](#) or with Tween20 [GS4200](#)

BSA [UPQ84170](#) (powder) or [UP900100](#) (convenient solution 30%)

BioBlock Saturating agent (in TBS) [N13650](#)

SeaBlock Saturating agent [UP40301](#) (no cross-reactivity with mammalian reagents)

RapidBlock saturating agent [DZ7330](#) (protein-free, 5min blocking step)

[FPlyte microplates](#)

*Other substrates for HRP:

chromogenic: DAB tablets [732310](#)

fluorogenic: ADHP ([39423A](#), kit [HS6241](#))

chemiluminogenic: UptiLight ECL Classic [UP996190](#), High Sensitivity [36349A](#), UltraSensitive [996201](#)

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