

**BioFX® TMB Super Slow One Component  
HRP Microwell Substrate  
Product Insert**

**Product number:**

TTMB-1000-01 (1000 mL)

Custom sizes available

**Intended use:**

The BioFX® TMB family has a broad range of kinetic rates with low background and improved signal-to-noise ratios that improve assay performance.

The BioFX TMB Super Slow One Component HRP Microwell Substrate contains 3,3',5,5'-tetramethylbenzidine (TMB) and is supplied as a ready-to-use solution. Peroxidase reacts with the substrate solution to produce a soluble blue reaction product that can be read at 370 nm or 620-650 nm for ELISA applications. Stop solutions can be added to prevent further color development and reading the absorbance values at the desired wavelengths.

- A soluble yellow product develops after stopping and stabilizing with a 450 nm stop solution.
- The developing soluble blue product can be stopped and stabilized by using a 650 nm stop solution.

**Product stability, storage and specifications:**

Product stability	Stable for 3 years from date of manufacture.
Storage	Product should be stored at 2-8°C. Exposure to direct sunlight and other UV sources should be avoided due to the light sensitive nature of the TMB molecule.
Notes	Please note the BioFX TMB substrates are shipped to customers at ambient temperatures. Extensive stability studies have shown that prolonged storage at ambient temperature will not affect the product quality or efficacy.

**Recommendations for use:**

Allow the substrate to equilibrate at room temperature (~25°C) prior to use.

**End-Point Assays:**

- 1) Add 100 µL of equilibrated substrate solution to each microwell.
- 2) Allow the substrate reaction to develop a blue soluble reaction by incubating for the optimal time determined for the assay.
- 3) The reaction should be stopped by using a 450 nm or 650 nm stop solution.
- 4) Measure the absorbance at 450 nm or 650 nm of each microwell within 1 hour.

**Kinetic Assays:**

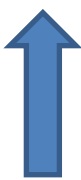
- 1) Add 100 µL of equilibrated substrate solution to each microtiter plate well.
- 2) Measure the absorbance at 650 nm at multiple time points.
- 3) Calculate the rate of absorbance change for each microwell.

**Additional considerations:**

The intensity of the reaction can be reduced by further dilution of the antibodies/conjugates used in the assay or to shorten the incubation time. Dilution of the substrate is not recommended.

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A wide range of kinetic rates with the same limit of detection are available to allow flexibility when developing an assay protocol.

<b>Kinetic Rate</b>	<b>BioFX® TMB Substrate:</b>
Faster    Slower	TMB Super Sensitive One Component HRP Microwell Substrate (TMBS)
	TMB Conductivity One Component HRP Microwell Substrate (TMBC)
	TMB One Component HRP Microwell Substrate (TMBW)
	TMB Slow Kinetic One Component HRP Microwell Substrate (TMSK)
	TMB Super Slow One Component HRP Microwell Substrate (TTMB)
	TMB Double Slow One Component HRP Microwell Substrate (TMDS)
	TMB Extended Range One Component HRP Microwell Substrate (TMBX)

**Related products:**

<b>In-Solution Protein Stabilizers &amp; Diluents:</b>
StabilZyme® HRP Conjugate Stabilizer (SZ02)
StabilZyme <i>SELECT</i> ® Stabilizer (SZ03)
StabilZyme® <i>NOBLE</i> Stabilizer BSA-Free (SZ04)
StabilZyme® Protein-Free Stabilizer (SZPF)
Assay Diluent-HAMA Blocker (SM01)
<b>Blockers/Stabilizers:</b>
StabilGuard® Immunoassay Stabilizer BSA-Free (SG01)
StabilCoat® Immunoassay Stabilizer (SC01)

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