

TMB Slow Kinetic 1-Component HRP Microwell Substrate

A one-component formulation suitable for all ELISAs using HRP.

TMB Slow Kinetic 1-Component HRP Microwell Substrate is suitable for use in all ELISAs where the target detection level is in the $\mu\text{g-ng/mL}$ range and horseradish peroxidase (HRP) is the conjugated detection enzyme. For assays requiring greater sensitivity, use TMB 1-Component HRP Microwell Substrate (catalog #6276) or TMB Super Sensitive 1-Component HRP Microwell Substrate (catalog #6275). TMB Slow Kinetic 1-Component HRP Microwell Substrate should not be used for membrane or immunohistochemical applications.

TMB Slow Kinetic 1-Component HRP Microwell Substrate is a one-component, ready-to-use formulation containing 3,3',5,5'-tetramethylbenzidine (TMB) in a mildly acidic buffer that does not contain aprotic solvents. TMB substrate is oxidized by the peroxidase enzyme to yield a soluble blue-green reaction product, which can be read at 370 nm or 620-650 nm. In endpoint assays, the reaction can be stopped by adding equal volumes of Stop Solution for TMB Microwell Substrates (catalog #6282). Addition of Stop Solution for TMB Microwell Substrates changes the chromagen color from blue-green to yellow, where it can be read at 450 nm, and concurrently stabilizes the yellow TMB product for one hour. Stopping the reaction will increase the sample absorbance value up to 3-fold. To avoid overdeveloping the TMB substrate reaction, the blue-green reaction product should be periodically monitored on an ELISA plate reader using 620-650 nm absorbance filter settings. When OD values reach approximately 0.7 units, the reaction should be stopped using Stop Solution for TMB Microwell Substrates.

For best results, the absorbance should be monitored and read before values exceed 2.5 OD units. The substrate should not be diluted. The intensity of the reaction can be reduced by further dilution of the antibodies/conjugates used in the assay or by shortening the incubation time.

TMB Slow Kinetic 1-Component HRP Microwell Substrate is ready to use at 1X; add 100 μL to each well. Best results are obtained by equilibrating the TMB substrate to room temperature (25°C) prior to use.

TMB SLOW KINETIC 1-COMPONENT HRP MICROWELL SUBSTRATE

Size	Catalog#
100 mL	6277

INSTRUCTIONS:

1. Run ELISA according to the specific protocol through the conjugate incubation step.
2. Wash the wells three or four times with 1X ELISA Wash Buffer (catalog #652) to remove any residual HRP-conjugate.
3. Bring TMB Slow Kinetic 1-Component HRP Microwell Substrate to room temperature; protect from light.
4. Pipette 100 μL TMB Slow Kinetic 1-Component HRP Microwell Substrate into each well of the plate.
5. Incubate TMB Slow Kinetic 1-Component HRP Microwell Substrate 10-60 minutes. Monitor the color intensity.
6. Read the plate at 370 nm or 620-650 nm and analyze. Alternatively, stop the reaction by adding 100 μL /well Stop Solution for TMB Microwell Substrates (catalog #6282) and read at 450 nm within 1 hour.

For more ELISA information and protocols, please visit www.immunochemistry.com.

SPECIFICATIONS:

- Colorless to light yellow liquid
- 1X ready to use
- Read absorbance for TMB at 370 nm or 620-650 nm
- Use Stop Solution for TMB Microwell Substrates to stabilize the reaction and read at 450 nm

STORAGE:

- 2-8°C
- Protect from light

SAFETY & USAGE:

- SDS available at immunochemistry.com
- Not for human or drug use
- For research use only



BRIGHT MINDS, BRIGHT SOLUTIONS.

ImmunoChemistry Technologies, LLC gratefully acknowledges the significant contributions made by one of its founders, Brian W. Lee, Ph.D in the development of this product, including the creation and illustration of its strategy and protocol.