

# TMB Substrate (Super Sensitive)

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

TMB Substrate (Super Sensitive) is suitable for use in all ELISAs using horseradish peroxidase (HRP) as the conjugated detection enzyme and requiring very sensitive detection levels or additional signal enhancement. TMB Substrate (Super Sensitive) can be used: to amplify the signal in ELISAs that require high dilutions of the test samples (e.g., 1:10,000); with samples that exhibit high steric hindrance; when the antibodies have a low binding capacity; or to shorten the incubation time of the assay. TMB Substrate (Super Sensitive) should not be used for membrane or immunohistochemical applications.

TMB Substrate (Super Sensitive) 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. The 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 (catalog# LS-M43). Addition of Stop Solution 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 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 Substrate (Super Sensitive) is ready to use at 1X; add 100  $\mu$ L to each well. Best results are obtained by equilibrating the TMB substrate to room temperature (25°C) prior to use.

### TMB Substrate (Super Sensitive)

Size	Catalog#
100 mL	LS-M36-100
1000 mL	LS-M36-1000

#### 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# LS-M27) to remove any residual HRP-conjugate.
3. Bring TMB Substrate (Super Sensitive) to room temperature; protect from light.
4. Pipette 100  $\mu$ L TMB Substrate (Super Sensitive) into each well of the plate.
5. Incubate TMB Substrate (Super Sensitive) 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  $\mu$ L/well Stop Solution (catalog# LS-M43) and read at 450 nm within 1 hour.

For more ELISA information and protocols, please visit [www.LSBio.com](http://www.LSBio.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 to stabilize the reaction and read at 450 nm

#### STORAGE:

- 2-8°C
- Protect from light

#### SAFETY & USAGE:

- SDS available upon request
- Not for human or drug use
- For research use only



2401 4th Avenue, Suite 900, Seattle, WA 98121

USA TOLL-FREE 1-866-819-4732 LOCAL 206-374-1102

[www.LSBio.com](http://www.LSBio.com)