

## TMB Substrate (Low Sensitivity)

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

TMB Substrate (Low Sensitivity) 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 Substrate (ELISA) (catalog# LS-M37) or TMB Substrate (Super Sensitive) (catalog# LS-M36). TMB Substrate (Low Sensitivity) should not be used for membrane or immunohistochemical applications.

TMB Substrate (Low Sensitivity) 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 (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 (Low Sensitivity) is ready to use at 1X; add 100  $\mu\text{L}$  to each well. Best results are obtained by equilibrating the TMB substrate to room temperature (25°C) prior to use.

### TMB Substrate (Low Sensitivity)

Size	Catalog#
100 mL	LS-M38-100

#### 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 (Low Sensitivity) to room temperature; protect from light.
4. Pipette 100  $\mu\text{L}$  TMB Substrate (Low Sensitivity) into each well of the plate.
5. Incubate TMB Substrate (Low Sensitivity) 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  $\mu\text{L}$ /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



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