

# TMB Super Sensitive 1-Component HRP Microwell Substrate (SUBS)

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

TMB Super Sensitive 1-Component HRP Microwell Substrate (SUBS) 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. SUBS 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. SUBS should not be used for membrane or immunohistochemical applications.

SUBS 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 for TMB Microwell Substrates (STOPT, catalog #6282). Addition of STOPT 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 STOPT.

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 Super Sensitive 1-Component HRP Microwell Substrate 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.

### 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.

## TMB SUPER SENSITIVE 1-COMPONENT HRP MICROWELL SUBSTRATE

Size	Catalog#
100 mL	6275
500 mL	6328
1 L	6329
5 L	6700
10 L	6330

### 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 SUBS to room temperature; protect from light.
4. Pipette 100  $\mu$ L SUBS into each well of the plate.
5. Incubate SUBS 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 STOPT (catalog #6282) and read at 450 nm within 1 hour.

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

### STORAGE:

- 2-8°C
- Protect from light

### SAFETY & USAGE:

- SDS available at [immunochemistry.com](http://immunochemistry.com)
- Not for human or drug use
- For research use only



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