Stop Solution for TMB Substrates

Stop and stabilize TMB microwell substrate reactions.

Stop Solution for TMB Substrates is a proprietary acidic formulation used to stop TMB microwell substrate reactions. It is suitable for all end-point ELISAs using a tetramethylbenzidine (TMB) substrate reaction for color development.

TMB substrates (e.g., catalog #6276, #6275, and #6277) are oxidized by peroxidase enzymes to yield a soluble blue-green reaction product. In endpoint assays, the reaction can be stopped by adding equal volumes of Stop Solution for TMB Substrates. Addition of Stop Solution for TMB 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 with Stop Solution for TMB Substrates.

For best results, the absorbance should be monitored and read before values exceed 2.5 OD units. If the yellow reaction product yields OD values above 2.5 units, it is recommended to dilute the antibodies/conjugates or shorten the incubation period.

Stop Solution for TMB Substrates is ready to use at 1X. Stop the reaction by adding an equal volume of Stop Solution for TMB Substrates to the substrate in every well of the plate.

Build a better assay with ELISA Solutions from ImmunoChemistry Technologies.

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.

STOP SOLUTION FOR TMB SUBSTRATES

Size	Catalog#
100 mL	6282
1 L	6343

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. Add TMB substrate to each well of the plate. For example, use 100 µL/well; protect from light.
4. Incubate the substrate 10-60 minutes. Monitor the color intensity.
5. Stop the reaction by adding an equal volume of Stop Solution for TMB Substrates to the substrate in every well of the plate, e.g., if each well contains 100 µL substrate, add 100 µL/well Stop Solution for TMB Substrates.
6. Read the plate at 450 nm within 1 hour.

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

SPECIFICATIONS:

- Clear, colorless liquid
- pH ≤ 2
- 1X ready to use
- Read absorbance at 450 nm

STORAGE:

- 25°C
- Refrigerated temperatures will not harm the reagent

SAFETY & USAGE:

- Contains Maleic acid at 3-7%
- SDS available at immunochemistry.com
- Not for human or drug use
- For research use only

ImmunoChemistry TECHNOLOGIES LLC