

## Product Information

### TMB ELISA Liquid Substrate

Catalogue Number	Size
ATR-E401	50 mL
ATR-E402	500 mL
ATR-E403	1000 mL

## Product Description

3,3',5,5'-Tetramethylbenzidine (TMB) serves as a chromogenic substrate ideal for employment in ELISA procedures involving horseradish peroxidase (HRP) conjugates. Upon interaction, this TMB-HRP reaction yields a soluble end-product appearing blue, suitable for spectrophotometric measurement at wavelengths of 370 or 655 nm. To stop the reaction, 2N sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) can be utilized, resulting in a yellow solution facilitating precise intensity assessment at 450 nm.

This substrate is supplied in a pre-prepared form, comprising TMB in a slightly acidic buffer, ready for peroxidase substrate application. Before peroxidase interaction, the substrate solution should exhibit a colorless to light bluish-green. The reaction between the substrate and peroxidase in microwell systems, such as ELISA assays, induces a blue reaction product. For endpoint assays, the reaction can be stopped using acid, leading to the formation of a yellow end-product.

## Applications

This substrate is suitable for peroxidase-based enzyme immunoassays, particularly ELISA procedures.

## Highlights

- **Ready-to-use formulation**
- **Free from DMF or DMSO**
- **Eliminates the need for additional reagents or filtration**

## Storage

Store the product at temperatures between 2-8 °C.

## Note

- Protect the substrate from direct sunlight or UV exposure. Keep it sealed in its original container until use to prevent light and air exposure. Reseal the container promptly after dispensing the reagent. While particulates may form over time, they do not compromise product integrity.
- This substrate is susceptible to contamination from various oxidizing agents. To prevent contamination and premature expiration, avoid direct contact with potential sources of contamination. Never pipette directly from the bottle; instead, pour the required amount into a separate tube for pipetting. Avoid returning excess substrate to the original container.

## Shipping

The Substrate is shipped with ice gel.

## Protocol

This product is a pre-prepared, single-component substrate for peroxidase and is supplied at the working dilution. Before reacting with peroxidase, the substrate solution should be colorless or light bluish-green. Interaction with peroxidase induces the formation of a blue reaction product. Recommended for ELISA procedures in microwell formats; not suitable for membrane applications or blotting due to its production of a soluble reaction product. Dilution of the substrate is not advised. To adjust the reaction intensity, consider diluting the antibodies or conjugates instead.

1. Allow the TMB ELISA Liquid Substrate to reach room temperature before use.
2. Dispense 50-100 µL of the TMB ELISA Liquid Substrate Solution into each well of the microplate.
3. Incubate the microplate at room temperature for 5-30 minutes until the desired color develops.

## Note

- Upon reaction with peroxidase, a blue product forms, detectable at either 370 nm or within the range of 620 nm - 655 nm.
- Higher concentrations of HRP may yield a greenish solution; in such cases, halt the reaction promptly to prevent the appearance of a green product. The presence of a precipitated product indicates excessive HRP levels

and necessitates optimization of experimental conditions.

4. For end-point assays, terminate the reaction by adding 2N sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) in an equal volume to that of the substrate reaction in each well.
5. Measure the absorbance of each well at 450 nm. The resulting yellow end-product, stable for at least one hour, can be subsequently read at 450 nm.

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## Precautions and Disclaimer

This product is intended for research and development (R&D) purposes only and is not suitable for use in drugs, diagnostic procedures, households, or other applications. When handling the product, always wear appropriate laboratory attire, including a lab coat, disposable gloves, and protective eyewear. When working with radioactive tracers, adhere to standard safety protocols for handling radioactive materials. For detailed safety information, refer to the relevant material safety data sheets (MSDSs), which are available online as PDF files or upon request via email ([info@atrmed.com](mailto:info@atrmed.com)). To the fullest extent permitted by law, ATR-MED Inc. disclaims liability for any special, incidental, indirect, punitive, multiple, or consequential damages arising from or related to the use of this document, including any associated products. By using this product, you acknowledge and agree to all terms and conditions outlined by ATR-MED. All trademarks mentioned herein are the property of ATR-MED unless otherwise specified.

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