WSE-7145

# EzELISA TMB

operating instructions

Aug. 06, 2025, 2nd edition

## 1. Precautions for safe use of this product

To use this product safely, please read this instruction manual carefully first. Please refrain from operating the product until you fully understand the contents of this instruction manual. This instruction manual describes only how to use this product for the specified purpose. Please refrain from using the product for purposes or in ways not described in this instruction manual. If you use the product for purposes or in ways not described in this instruction manual, you are solely responsible for all necessary safety measures and unforeseen circumstances. Also, please carefully read and understand the instruction manuals of any devices you will be using at the same time.

## 2. Purpose of use

This product contains a chromogenic substrate solution, and a reaction stop solution for detecting HRP (horseradish peroxidase)-labeled antibodies used in ELISA. It cannot be used as a chromogenic substrate for Western blotting.

## 3. Product configuration

| Name | Volume | Quantity |
|---|---|---|
| **TMB Solution** | 200 mL | 1 bottle |
| **STOP Solution** | 200 mL | 1 bottle |

At 100 µL per well, sufficient for 20 × 96-well plates.

## 4. Composition

| Name | Main component |
|---|---|
| **TMB Solution** | 3,3',5,5'- Tetramethylbenzidine hydrogen peroxide |
| **STOP Solution** | Phosphoric acid |

This product contains substances subject to notification that exceed the exemption quantities specified under the PRTR Act, the Poisonous and Deleterious Substances Control Act, and the Industrial Safety and Health Act. For details, please download and refer to the SDS for this product from the ATTO website (https://www.atto.co.jp).

## 5. Storage

- This reagent should be stored in a refrigerator at 2 to 10 °C, away from direct sunlight. If unopened, it is stable within the expiration date (approximately one year from the date of manufacture).
- **TMB Solution** will turn brown when it comes into contact with metal ions. Be careful not to mix it with tap water, etc.
- **STOP Solution** can be stored at room temperature (15-30 °C). Please handle it with care as it contains phosphoric acid.

## 6. Disposal method

- Dispose of each reagent in accordance with the disposal method of your institution. Please be careful when disposing of **STOP Solution,** as it contains phosphoric acid.
- Bottle Material Body: Polyethylene
  Lid: Polypropylene

## 7. Items required other than this product

- ELISA plates
- ELISA Reagents
  (Standards, blocking agents, cleaning solutions, etc. )
- Primary antibodies, HRP -labeled secondary antibodies, etc.
- Measuring devices such as plate readers

## 8. Precautions for use

- This product is ready-to-use and does not require any reagent addition, mixing, or dilution.
- Please note that the **TMB solution** will turn brown when it comes into contact with metal ions.
- **STOP solution** contains phosphoric acid, so please handle it with care.
- Ensure that the **TMB Solution** and the **STOP Solution** are kept separate and do not come into contact before use.
- Use ELISA kits, antibodies, and other reagents as per the manufacturer's recommended procedures and concentrations.

## 9. How to use

1. Bring **TMB Solution** and **STOP Solution** to room temperature before use. The amount of each reagent required per 96-well plate is approximately 10 mL.

   *The required amount of each reagent is 100 µL/well.

2. Prepare an ELISA plate before detection, after antibody reaction and washing.

3. Add 100µL of **TMB Solution** to each well.

   *Be careful not to touch the tip of the pipette tip to the plate or well.

4. The plate will turn blue immediately after the reaction, and signal detection will begin. If measuring changes over time without stopping the reaction, measure the absorbance at 620-650nm using a plate reader.

   *With high-concentration samples, precipitation may be observed if the reaction time is prolonged.

5. The reaction time depends on the sample but incubate in the dark for 5-30 minutes.

   *Prolonged reaction times may result in high background levels.

6. Stop the reaction by adding 100µL of **STOP Solution** to each well. The plate will turn yellow after addition.

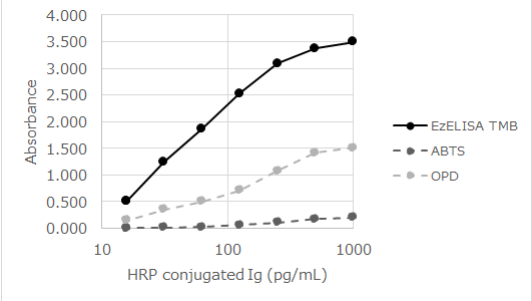
   ***STOP Solution** contains phosphoric acid, so handle with care.

7. Immediately after stopping the reaction, measure the absorbance at 450 nm using a plate reader. To correct the background between wells, measure the absorbance at a reference wavelength of 570 nm (between 540 and 650 nm) and subtract it from the absorbance at 450 nm.

## 10. Reference materials

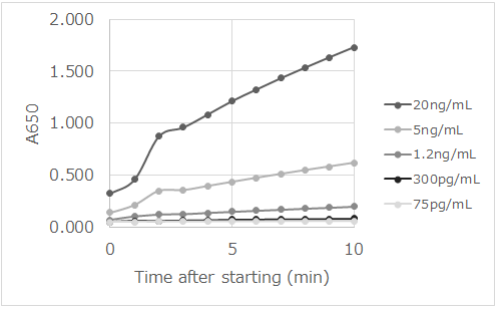
Even with the same protocol, slight differences in technique can significantly affect results. Please refer to the ATTO website for various experimental know-how downloads.
https://www.atto.co.jp/

## 11. Measurement data



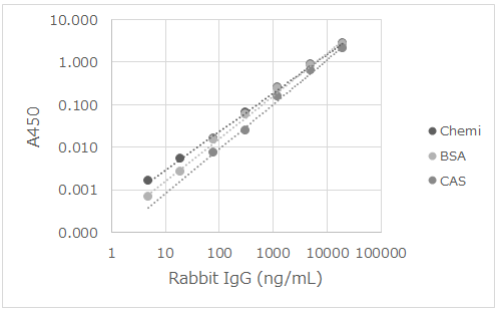

**Comparison with HRP detection substrate**

These are the results of detecting a dilution series of HRP-labeled antibodies with various chromogenic substrates. Compared to ABTS and OPD, **EzELISA TMB** demonstrates higher detection sensitivity, signal intensity, and S/N ratio.



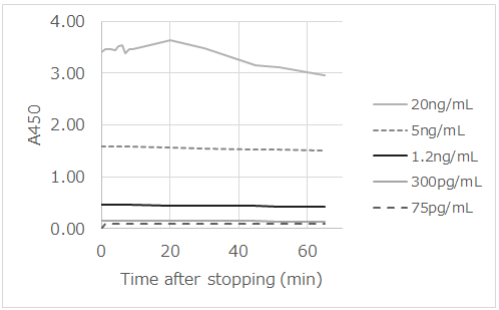

**EzELISA TMB Reaction Kinetics**

The graph shows the change in absorbance at 650 nm measured over time when various concentrations of HRP-labeled antibodies were detected with **EzELISA TMB**.




**Detection sensitivity and blocking**

A plate coated with a dilution series of rabbit antibodies was blocked with **EzBlock Chemi** (Chemi), **EzBlock BSA** (BSA), and **EzBlock CAS** (CAS), and then detected by Direct ELISA. This shows that **EzELISA TMB** can detect down to a few pg/mL.




**EzELISA TMB color stability**

HRP-labeled antibodies at various concentrations were detected with **TMB Solution**, and **STOP Solution** was added after 5 min to terminate the reaction. Absorbance at 450 nm was monitored over time. In the 20 ng/mL sample, precipitation began after reaction termination, causing a gradual decrease in absorbance from around 20 min. Precipitation may occur in high-concentration samples.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