TMB ELISA Liquid Substrate

Product Information

TMB ELISA Liquid Substrate

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

Product Description

ATR-MED® TMB ELISA Liquid Substrate is a ready-to-use chromogenic substrate formulated for enzyme-linked immunosorbent assays (ELISAs) utilizing horseradish peroxidase (HRP) conjugates. The substrate contains 3,3',5,5'-tetramethylbenzidine (TMB) in a mildly acidic proprietary buffer, optimized for peroxidase-mediated reactions. Upon interaction with HRP, TMB undergoes oxidation, producing a soluble blue reaction product detectable spectrophotometrically at 370 nm or 620–655 nm. For endpoint assays, the reaction can be terminated with 2N sulfuric acid (H_2SO_4), yielding a stable yellow end-product measurable at 450 nm with enhanced sensitivity. The substrate is supplied as a single-component, pre-diluted solution, eliminating the need for additional reagents, dilution, or filtration. Prior to reaction with HRP, the solution appears colorless to faintly bluish-green. The formulation is free of dimethylformamide (DMF) and dimethyl sulfoxide (DMSO), ensuring compatibility with standard ELISA protocols. The substrate is designed for microwell-based ELISA systems and is not suitable for membrane-based applications or blotting due to the soluble nature of the reaction product. This product delivers consistent, sensitive, and reproducible colorimetric signals for quantitative and qualitative immunoassays.

Applications

- Peroxidase-based enzyme immunoassays, particularly ELISAs
- Quantification of HRP-conjugated antibodies or analytes in microwell formats

Highlights

- **Ready-to-Use**: Pre-formulated solution requires no additional preparation or dilution.
- **High Purity**: Free of DMF and DMSO, minimizing interference in sensitive assays.
- **Versatility**: Suitable for both kinetic (370 nm or 620–655 nm) and endpoint (450 nm) measurements.
- **Stability**: Maintains integrity despite potential particulate formation over time.

Storage

Store at 2–8°C in the original container to protect from light and air exposure. Reseal immediately after dispensing to maintain stability. Stable for at least 12 months under recommended conditions. Particulate formation may occur over time but does not affect performance.

Shipping

Shipped on gel ice packs at ≤0°C to ensure stability. Transfer immediately to a 2–8°C refrigerator upon receipt.

Protocol

ATR-MED® TMB ELISA Liquid Substrate is a pre-diluted, single-component solution optimized for HRP-based ELISAs in microwell formats. Dilution of the substrate is not recommended; adjust reaction intensity by optimizing antibody or conjugate concentrations instead.

1. Allow the TMB ELISA Liquid Substrate to equilibrate to room temperature (20–25°C) before use to ensure consistent performance.
2. Dispense 50–100 µL of the substrate into each well of a microwell plate using a clean pipette tip to avoid contamination. Do not pipette directly from the bottle; pour the required volume into a separate tube for dispensing.
3. Incubate at room temperature (20–25°C) for 5–30 minutes, depending on the desired color intensity, monitored visually or spectrophotometrically.

   Note: The reaction produces a blue product measurable at 370 nm or 620–655 nm. High HRP concentrations may yield a greenish hue, indicating the need to stop the reaction promptly or optimize HRP levels to prevent precipitation.
4. For endpoint assays, terminate the reaction by adding an equal volume (50–100 µL) of 2N sulfuric acid (H_2SO_4) to each well, converting the blue product to a stable yellow end-product.
5. Measure absorbance at 450 nm within 1 hour of stopping the reaction for optimal stability and sensitivity.

Important Notes

1. Contamination Prevention

To ensure assay integrity and prevent premature substrate oxidation:

- Avoid exposure to oxidizing agents (e.g., bleach, peroxides) or other contaminants.

- Use dedicated pipettes and sterile, nuclease-free pipette tips.
- Pour substrate into a separate tube for dispensing; do not return unused substrate to the original container.
- Work in a clean environment, preferably under a laminar flow hood, to minimize contamination.

2. Reaction Optimization

- **Color Development**: Monitor incubation time to achieve desired signal intensity. Over-incubation or excessive HRP can lead to precipitation or non-specific color changes (e.g., green hue).
- **HRP Concentration**: Optimize antibody or conjugate dilutions to avoid excessive HRP activity, which may cause substrate precipitation or signal saturation.
- **Spectral Measurements**: Use 370 nm or 620–655 nm for kinetic assays; 450 nm for endpoint assays after acid stop for maximum sensitivity.

3. Substrate Handling

- **Light Sensitivity**: Protect from prolonged exposure to sunlight or UV light to prevent degradation of TMB.
- **Particulate Formation**: Minor particulates may form during storage but do not compromise performance. Gently mix before use; avoid vigorous shaking to prevent bubble formation.

Precautions and Disclaimer

This product is designated for research and development purposes only and is not intended for therapeutic, diagnostic, household, or other non-research applications. Handle using standard laboratory protective equipment, including lab coats, disposable gloves, and safety goggles. When using radioactive materials in conjunction with this substrate, adhere to institutional radiation safety protocols. Minimize light exposure to prevent TMB degradation. Comprehensive safety data are available in the Material Safety Data Sheets (MSDSs) at www.atrmed.com or via email request to info@atrmed.com. To the maximum extent permitted by applicable law, ATR-MED Inc. disclaims liability for special, incidental, indirect, punitive, or consequential damages arising from the use of this product or associated documentation. Product use constitutes acceptance of ATR-MED's terms and conditions. All trademarks are owned by ATR-MED unless otherwise specified.

Limited Product Warranty

ATR-MED® guarantees that at the time of quality release or retest, this product conforms to the specifications herein, pursuant to the General Terms and Conditions of Sale at www.atrmed.com/terms-and-conditions.html. For warranty inquiries, contact support at www.atrmed.com/support. Users must independently verify product suitability for their applications. Additional terms may be included on invoices or packing slips.

Trademarks

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Problem	Potential Cause(s)	Solution(s)
No or Weak Color Development	Insufficient HRP activity	Verify HRP conjugate activity; increase conjugate concentration or incubation time.
	Substrate degradation	Use fresh substrate; ensure proper storage at 2–8°C and protection from light.
	Contamination with inhibitors	Avoid contact with oxidizing agents; use clean pipettes and sterile tips.
Greenish Reaction Product	Excessive HRP concentration	Reduce HRP conjugate concentration or shorten incubation time.
	Delayed reaction termination	Stop reaction promptly with 2N H_2SO_4 when desired intensity is reached.
Precipitation in Wells	Excessive HRP or over-incubation	Optimize HRP conjugate dilution and incubation time to prevent substrate overuse.
High Background Signal	Non-specific binding	Optimize blocking and washing steps; reduce antibody/conjugate concentrations.
	Substrate contamination	Avoid pipetting directly from bottle; use fresh substrate and clean equipment.
Inconsistent Absorbance	Inconsistent pipetting	Use calibrated pipettes and ensure uniform dispensing volumes.
	Temperature variability	Equilibrate substrate to room temperature; ensure consistent incubation conditions.
	Bubbles in wells	Centrifuge plate briefly or tap gently to remove bubbles before reading.