

TMB Substrate Chromogenic Kit (ELISA, HRP Chromogenic)

Cat. No.: T1509497 | Pack size: 2 x 50 mL / 2 x 100 mL | Storage: 2-8 °C, protected from light

Overview

3,3',5,5'-Tetramethylbenzidine (TMB) is an excellent chromogenic reagent for enzyme immunoassays. It reacts with peroxidase in the presence of oxidant and buffer to produce a distinct blue product that can be observed easily.

This TMB Substrate Chromogenic Kit for ELISA HRP chromogenesis contains TMB Chromogenic Solution, TMB Buffer and TMB Oxidant. It offers high sensitivity, good stability after chromogenic termination and low background.

Key Features

- Ready kit format for ELISA HRP chromogenesis
- Working solution prepared at A:B:C = 5000:5000:4
- Blue reaction product turns yellow after acid stop solution
- Measure absorbance at 450 nm within 30 minutes after termination

Product Information

Item	Description
Synonyms	TMB Substrate Chromogenic Kit
Specifications & Purity	BioReagent
Grade	BioReagent
Application	ELISA
Sensitivity	Light-sensitive
Stability & Storage	Each component has a shelf life of 1 year under corresponding storage conditions.
Storage Conditions	Store at 2-8 °C, protected from light
Shipping	Wet ice

Contents & Storage

Cat. No.	Component / Product	Size	Storage
T1509497A	TMB Chromogenic Solution	50 mL / 100 mL	2-8 °C, protect from light
T1509497B	TMB Buffer	50 mL / 100 mL	2-8 °C
T1509497C	TMB Oxidant	100 µL / 2 x 100 µL	2-8 °C, protect from light

Materials Required But Not Supplied

Item	Recommended Specification	Purpose
Personal protective equipment	Lab coat, disposable gloves and eye protection	Safe handling and contamination control
Microplate reader	450 nm	Absorbance reading
Microtiter plate or 96-well plate	ELISA-compatible	Reaction vessel
Incubator	Optional, RT or 37 °C	Chromogenic reaction
Stop solution	Optional 1 M hydrochloric acid or sulfuric acid	Terminate reaction
Clean container	Rinsed repeatedly with pure water	Working solution preparation

Preparation Before Use

1. Bring reagents to room temperature before preparing working solution.
2. Prepare TMB chromogenic working solution by mixing Reagent A, B and C at a volume ratio of 5000:5000:4 in a clean container.
3. Use the working solution after it reaches room temperature.

Protocol

Operating Procedures (For Reference Only)

1. Prepare TMB chromogenic working solution by mixing Reagent A, B and C at A:B:C = 5000:5000:4.
2. After adding HRP conjugate and incubating as required by the ELISA method, wash the plate 3-5 times.
3. Add 100 μ L TMB chromogenic working solution to each well.
4. Incubate at room temperature (15-25 °C) or 37 °C for 10-30 minutes.
5. Optional termination: add an equal volume of 1 M hydrochloric acid or sulfuric acid to stop the reaction; the reaction solution changes from blue to yellow.
6. Read absorbance at 450 nm within 30 minutes.

Expected Results

Step	Expected Result
HRP/TMB chromogenesis	Blue color development
After acid stop solution	Yellow color suitable for 450 nm reading
Low background	OD of substrate solution at 450 nm generally below 0.05

Storage & Handling

Store as indicated on the product label: 2-8 °C, protected from light.

Shelf life: Each component has a shelf life of 1 year under corresponding storage conditions.

Safety & Precautions

1. The amount of TMB Oxidant provided exceeds actual usage; use strictly according to the specified ratio.
2. TMB Oxidant is volatile; store sealed to avoid activity loss and use promptly after opening.
3. TMB Oxidant is corrosive; avoid direct contact with skin, hair and other body surfaces.
4. High reaction background or precipitation indicates an excessively intense TMB substrate reaction.
5. Reaction termination is optional; if no stop solution is added, detect immediately after chromogenesis.

6. Concentrated hydrochloric acid and sulfuric acid are volatile/corrosive; handle with care.
7. To avoid precipitation, read immediately after termination or further dilute primary antibody and/or HRP conjugate and repeat the experiment.
8. Wear a lab coat and disposable gloves during operation.

Quality Control

QC Item	Method	Acceptable Range
Background check	Measure substrate solution at 450 nm	OD generally less than 0.05.
Color development	HRP-positive control	Blue develops during reaction and turns yellow after stop solution.
Storage integrity	Receipt and storage inspection	Components stored at 2-8 °C protected from light.

Troubleshooting

Issue	Possible Causes	Corrective Action
High background	Excessive HRP/substrate reaction or inadequate washing	Reduce antibody/HRP conjugate concentration, shorten development or improve washing.
Precipitation after stop	Reaction too intense or delayed reading	Read immediately after termination or dilute antibody/HRP conjugate.
Weak color	Insufficient HRP activity or short incubation	Verify conjugate activity and extend development within 10-30 min.
Oxidant loss	Oxidant left unsealed	Keep TMB Oxidant sealed and use promptly after opening.

Recommended Applications

ELISA chromogenic substrate · HRP detection · enzyme immunoassay color development · 450 nm microplate reading

Contact & Global Offices

Whether you have a technical question, need help with a quotation, or want to inquire about an order, our regional teams are ready to assist. Please contact the office for your region; for general inquiries, the North American office is the corporate primary.

NORTH AMERICAN SALES, SUPPORT & GENERAL INQUIRIES

Aladdin Scientific Corporation

14078 Meridian Parkway, Riverside, CA 92518, USA

Phone: 1-833-552-7181

Sales: sales@aladdinsci.com

Customer Service: custserv@aladdinsci.com

EU SALES, LOGISTICS & LOCALIZED SUPPORT

Aladdin Biochem Deutschland GmbH

Westring 2, 33142 Büren, Germany

Phone: +02951 9383958

Support: support.eu@aladdinsci.com

Limitations & Disclaimer

- For Research Use Only (RUO). Not for use in human or animal diagnostics, therapeutics, or in vivo applications. Not for food, cosmetic, or household use.
- This product is not a CE-marked in vitro diagnostic device under IVDR (EU) 2017/746 and is not an FDA-cleared device under 21 CFR. Use is restricted to verified businesses, institutions, and qualified professionals for research and development purposes.
- Where any kit component is classified as hazardous under CLP (EC) 1272/2008 or OSHA HCS (29 CFR 1910.1200), the product Safety Data Sheet (SDS) takes precedence over this document for handling, storage, and disposal information.
- Performance depends on sample type, sample condition, handling, and operator technique. Users are responsible for validating the product for their specific application.
- Aladdin product labels, SDS, COA, and approved specifications take precedence over this document. If product formulation, label, SDS, storage conditions, pack size, or quality specifications change, this document would be reviewed and reissued.