CYTODIAGNOSTICS

PRODUCT DATA SHEET

CytoBlue Substrate

Description

3,3',5,5'-Tetramethylbenzidine (TMB) is a chromogenic substrate that can be used for assay procedures, such as colorimetric ELISA. Upon reaction with horseradish peroxidase (HRP) in the presence of hydrogen peroxide (H_2O_2), the colourless liquid turns blue. When the endpoint is reached, the HRP-TMB reaction is stopped with the addition of an acid, which turns it into a yellow-coloured product. The colour intensity of the reaction product is measured at 450 nm.

Feature

CytoBlue Substrate is a convenient, ready-to-use chromogenic substrate for HRP-based immunoassays. This single-bottle formulation combines TMB and H_2O_2, offering long-term stability and high enzymatic activity. This product is safe for laboratory use, containing no hazardous substances above regulatory reporting levels, and complies with standard safety regulations.

Product	Volume
EL-C10-50ML	50 mL
EL-C10-200ML	200 mL
EL-C10-500ML	500 mL
EL-C10-1L	1 L

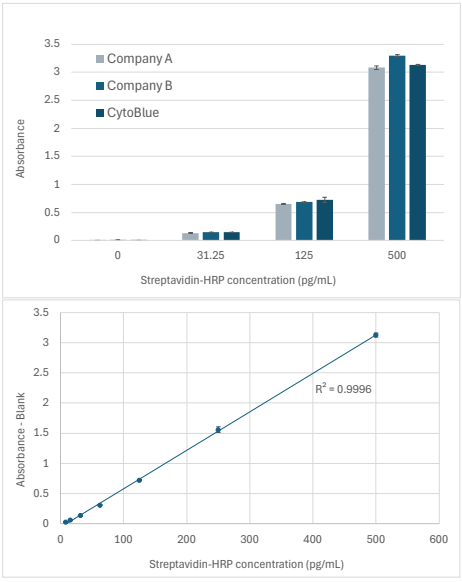

Figure: (Top) Comparison of CytoBlue Substrate with TMB substrates from two other sources, showing efficient color development with minimal standard deviation and low background noise. (Bottom) Titration of streptavidin-HRP tested in TMB reaction using CytoBlue Substrate, demonstrating a linear, concentration-dependent response.

Directions for Use

CytoBlue Substrate is ready-to-use; no mixing or pre-warming to room temperature is required.

Prepare the plate: Thoroughly wash the microplate to remove all unbound enzyme conjugate.

Add substrate: Dispense 100–150 µL of CytoBlue Substrate per well. Use a multichannel pipette if necessary.

Incubation: Let the plate sit at room temperature. Keep monitoring once the color development begins.

Note: Do not dilute CytoBlue Substrate. If absorbance is too high for assay, consider: adjusting incubation times, modifying concentration or volume of other assay reagents.

For Kinetic Assays:

After incubation, gently shake the plate to mix the color evenly. Measure absorbance at 650 nm (range 630–650 nm). For dual-wavelength readings, subtract absorbance at 490 nm from 650 nm.

For Endpoint Assays:

Red Stop Solution: Add 100–150 µL per well after the recommended incubation. Gently shake to mix, then read absorbance at 650 nm within 2 hours. Subtract 490 nm if using dual-wavelength mode.

Acid Stop Solution (1N H_2SO_4): Add 100–150 µL per well; the solution turns yellow. Gently shake, measure absorbance at 450 nm within 2 hours (or within 30 minutes if not using 1N H_2SO_4). For dual-wavelength readings, subtract 650 nm from 450 nm.

Recommended Handling

TMB-based substrates, including CytoBlue, require careful handling and storage to maintain performance. Please follow these precautions:

Light Sensitivity: CytoBlue is highly sensitive to light. Avoid direct sunlight and prolonged exposure to bright light.

Storage Containers: Store the substrate in high-quality amber glass or plastic bottles (HDPE amber bottles recommended) to protect it from light.

Contact Materials: Avoid contact with metals, as ions like iron can oxidize the substrate and increase background signal. Use only plastic or glass when handling.

Pipetting: Do not pipette directly from the main bottle to prevent contamination. Pour only the required amount into a separate container for use.

Bottle Handling: Always replace the cap promptly and avoid leaving the bottle open for extended periods.

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CYTO DIAGNOSTICS

Storage

This product should be stored at 2-8°C. Do not freeze. If stored and handled as specified, CytoBlue Substrate is stable for at least 12 months.

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Not for resale without express authorization. Please consult the Material Safety Data Sheet available online at www.cytodiagnostics.com for information regarding hazards and safe handling procedures.

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