KPL TMB Microwell Peroxidase Substrate System

Catalog No.	Size
5120-0047 (50-76-00)	600 mL
5120-0050 (50-76-03)	2700 mL
5120-0053 (50-76-11)	200 mL

DESCRIPTION

TMB Microwell Peroxidase Substrate System is a 2 component system which develops a deep blue color when reacted with horseradish peroxidase conjugates in ELISA. It is not recommended for blotting or immunohistochemical assays.

FORM

5120-0047 (50-76-00) consists of the following:
- 3 x 100 mL KPL TMB Peroxidase Substrate
- 3 x 100 mL KPL Peroxidase Substrate Solution B

5120-0050 (50-76-03) consists of the following:
- 3 x 450 mL KPL TMB Peroxidase Substrate
- 3 x 450 mL KPL Peroxidase Substrate Solution B

5120-0053 (50-76-11) consists of the following:
- 1 x 100 mL KPL TMB Peroxidase
- 1 x 100 mL KPL Peroxidase Substrate Solution B

STORAGE/STABILITY

Store both components at 2-8°C. Stable for a minimum of 1 year from date of receipt when stored at 2-8°C. The KPL TMB Peroxidase Substrate solution may develop a yellow tinge over time which does not affect product performance.

CONTENT

KPL TMB Peroxidase Substrate contains 3,3',5,5'-tetramethylbenzidine at a concentration of 0.4 g/L in an organic base. KPL Peroxidase Substrate Solution B contains H202 at a concentration of 0.02% in a Citric Acid buffer.

USE

Preparation:

Mix equal volumes of KPL TMB Peroxidase Substrate and KPL Peroxidase Substrate Solution B in a clean, preferably HDPE, polypropylene or glass container immediately prior to use. Substrate solution should remain clear. Warm to room temperature before use.

Substrate Development:

Following incubation with peroxidase labeled conjugate, wash plate thoroughly. Add 100 µL prepared substrate solution to each well. As the color develops, tap gently to mix. Incubation times will vary depending on your assay.

To Stop Reaction:

Stop reaction by adding an equal volume of stop solution to the microwell plate. This will halt color development and will turn the KPL TMB substrate yellow.
(See Recommended Stop Solution).

To Read Reaction:

After stopping, read at a wavelength of 450 nm. Stopped reaction should be read within 30 minutes.

When to Stop Substrate Reaction:

Upon addition of stop solution, absorbance values increase 2 – 3-fold. The point at which the substrate reaction is stopped is often determined by the ELISA reader. The O.D. values of the plate should be monitored and the reaction stopped before positive wells are no longer recordable.

To Reduce Substrate Intensity:

High background and/or precipitate in the wells are a sign of overreaction with TMB. To reduce the intensity of the substrate reaction, dilution of the primary antibody and/or conjugate is recommended. Dilution of the substrate is not recommended.

ABSORBANCE MEASUREMENTS

Kinetic Assays: The TMB substrate produces a blue color upon reaction with peroxidase. Read at a wavelength of 620 - 650 nm.
Endpoint Assays: The addition of 100 µL (or an equal volume) of stop solution to the microwell plate will halt color development and will turn the TMB substrate yellow. Read at a wavelength of 450 nm. Stopped reactions should be read within 30 minutes.

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RECOMMENDED STOP SOLUTION

The addition of an equal volume (100 µL) of 1M Phosphoric Acid (H_3PO_4) provides the most stable endpoint color. Other commonly used acids (HCl, H_2SO_4) are less efficient in stopping color development.

NOTE: The addition of KPL TMB Membrane Enhancer, 5420-0026 (50-77-01), allows the KPL TMB Microwell Substrate to be used on membranes.

PRODUCT SAFETY AND HANDLING

See SDS (Safety Data Sheet) for this product.

RELATED PRODUCTS	CAT. NO.
KPL Milk Diluent/Blocking Solution Concentrate	5140-0011 (50-82-01)
KPL BSA Diluent/Blocking Solution	5140-0006 (50-61-00)
KPL Detector™ Block	5920-0004 (71-83-00)

The product listed herein is for research use only and is not intended for use in human or clinical diagnosis.