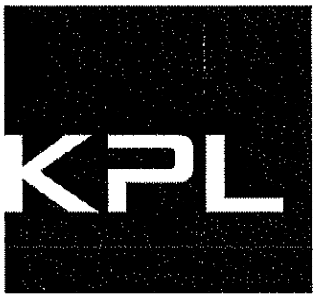
SureBlue™ TMB Microwell Peroxidase Substrate (1-Component)



Catalog No.	Size
52-00-00	100 mL
52-00-01	100 mL
52-00-02	400 mL
52-00-03	1000 mL
52-00-04	5000 mL
52-00-05	19500 mL

DESCRIPTION

SureBlue™ TMB Microwell Peroxidase Substrate (1-Component) develops a deep blue soluble product when reacted with horseradish peroxidase labeled conjugates in microwell plates or tubes. SureBlue™ is suitable for qualitative or quantitative microwell immunoassays. It is not suitable for membrane or immunohistochemical staining. (See KPL's catalog for appropriate substrates.)

APPEARANCE/BACKGROUND

The expected appearance of the solution is colorless to light amber. KPL's QC specification for background of unreacted substrate at 650 nm is ≤ 0.03 OD

FORM/STORAGE/STABILITY

Liquid. Store at 2 - 8°C. Do not freeze. Stable for a minimum of 24 months from date of manufacture when stored at 2 - 8°C.

CONTENT

SureBlue™ TMB Microwell Peroxidase Substrate (1-Component) contains 3,3',5,5'-tetra-methylbenzidine in an acidic buffer.

- 52-00-01 contains 1 x 100 mL SureBlue™ TMB Microwell Peroxidase Substrate (1-Component)
- 52-00-02 contains 4 x 100 mL SureBlue™ TMB Microwell Peroxidase Substrate (1-Component)
- 52-00-03 contains 1 x 1 Liter SureBlue™ TMB Microwell Peroxidase Substrate (1-Component)
- 52-00-04 contains 1 x 5 Liter SureBlue™ TMB Microwell Peroxidase Substrate (1-Component)
- 52-00-05 contains 1 x 19.5 Liter SureBlue™ TMB Microwell Peroxidase Substrate (1-Component)

RECOMMENDED HANDLING

SureBlue™ TMB Microwell Peroxidase Substrate (1-Component) is extremely sensitive to certain handling and storage conditions.

- Avoid exposure to sunlight, air and extreme temperatures, however exposure to indirect laboratory lighting for 24 hours is not harmful.
- Never pipette directly from bottle. Pour out needed amount into a plastic reservoir. Do not return excess SureBlue™ to the primary container.
- Avoid exposure to silica-based materials including borosilicate glass; metals; bacterial contamination or other oxidizing agents.
- Do not refilter as this may destabilize the dye complex and result in the development of background color.
- Redispense into amber Nalgene HDPE and LDPE bottles ONLY. Do not redispense into glass bottles as this may compromise substrate performance.
- HDPE and LDPE bottles should be utilized as they are received directly from the manufacturer; washing of bottles is not recommended.
- Redispense substrate using Cole-Parmer C-Flex tubing and do not reuse tubing. Avoid redispensing with all other tubings including silicone, latex and Tygon.
- Gravity fill or the use of a peristaltic pump is advised.
- Avoid substrate contact with any metallic surfaces.
- For further details concerning the redispensing of this product, please contact KPL's Technical Service Department at 800-638-3167 or 301-948-7755 ext. 233.

USE

Warm to room temperature before use. Solution is ready to use.

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

To Stop Reaction: For optimal performance, stop reaction by adding equal volume of 1N HCl or TMB Stop Solution to the microwell plate. This will halt color development and will turn the SureBlue™ substrate yellow (See Recommended Stop Solutions.).

To Read Reaction: After stopping, read at a wavelength of 450 nm. Stopped reaction should be read within 1 – 2 hours.

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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 OD values of the plate should be monitored and the reaction stopped before positive wells are no longer readable.

To Reduce Substrate Intensity: High background and/or precipitate in the wells are a sign of over-reaction with SureBlue™ TMB. To reduce the intensity of the substrate reaction, further dilution of the primary antibody and/or HRP labeled conjugate is recommended. Dilution of the substrate is not recommended.

ABSORBANCE MEASUREMENTS

Kinetic Assays:
The SureBlue™ TMB Microwell Peroxidase Substrate (1-Component) 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 SureBlue™ TMB Microwell Peroxidase Substrate (1-Component) yellow. Read at a wavelength of 450 nm. Stopped reactions should be read within 30 minutes.

RECOMMENDED STOP SOLUTIONS

For best results, we recommend 1N HCl or KPL's TMB Stop Solution.

TMB Stop Solution	4 x 100 mL Catalog No. 50-85-05
	1 x 1000 mL Catalog No. 50-85-06

REFERENCES

Porstman, T., and Kiessig, S.T. (1992). J. Immunol. Methods 150: 521.

PRODUCT SAFETY AND HANDLING

See MSDS (Material Safety Data Sheet) for this product.

RELATED PRODUCTS

TMB BlueSTOP Solution	Catalog No. 50-85-30
	Catalog No. 50-85-41
TMB Stop Solution	Catalog No. 50-85-05
	Catalog No. 50-85-06
Wash Solution Concentrate	Catalog No. 50-63-00
BSA Diluent/Blocking Solution Concentrate	Catalog No. 50-61-00
Coating Solution Concentrate	Catalog No. 50-84-00

See KPL's catalog for a list of antibodies, conjugates, substrates and complete systems for ELISA, Western blotting and immunohistochemical applications.

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