

# SureBlue Reserve™ TMB Microwell Peroxidase Substrate (1-Component)



| Catalog No. | Size    |
|-------------|---------|
| 53-00-01    | 100 mL  |
| 53-00-02    | 400 mL  |
| 53-00-03    | 1000 mL |
| 53-00-04    | 5000 mL |

## DESCRIPTION

SureBlue Reserve TMB Microwell Peroxidase Substrate (1-Component) contains 3,3',5,5' tetramethylbenzidine in a mildly acidic buffer. It develops a deep blue soluble product when reacted with horseradish peroxidase labeled conjugates in microwell plates or tubes. SureBlue Reserve provides more sensitivity compared to standard SureBlue TMB substrate. It is suitable for qualitative or quantitative microwell immunoassays and not recommended for membrane or immunohistochemical applications. (See KPL's catalog for appropriate substrates).

## FORM/STORAGE/STABILITY

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

## CONTENT

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

- 53-00-01 contains 1 x 100 mL SureBlue Reserve TMB Microwell Peroxidase Substrate (1-Component)
- 53-00-02 contains 4 x 100 mL SureBlue Reserve TMB Microwell Peroxidase Substrate (1-Component)
- 53-00-03 contains 1 x 1000 mL SureBlue Reserve TMB Microwell Peroxidase Substrate (1-Component)

## APPEARANCE/BACKGROUND

The expected appearance of the solution is colorless to very light yellow. KPL's QC specification for background of unreacted substrate at 650 nm is  $\leq 0.03$  Au.

## RECOMMENDED HANDLING

SureBlue Reserve 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 8 hours is not harmful. A pale blue color may become apparent after 4 – 5 hours of exposure to indirect laboratory lighting, but this will not affect product performance.
- Never pipette directly from bottle. Pour out needed amount into a plastic reservoir. Do not return excess SureBlue Reserve 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 Platinum Cured Silicone tubing and do not reuse tubing. Avoid redispersing with all other tubings.
- Gravity fill or the use of a peristaltic pump is advised.
- Avoid substrate contact with any metallic surfaces.
- For further details concerning the redispersing 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  $\mu$ L substrate solution to each well. As the color develops, tap gently to mix. Incubation times will vary depending on your assay.

## Over-reaction

Precipitate in the wells after stopping is a sign of over-reaction with SureBlue Reserve. Precipitate usually takes 10 – 20 minutes to develop so reading the plate immediately after adding the stop solution is recommended. 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.

## To Stop Reaction

For optimal performance, stop reaction by adding equal volume of 1N HCl, 0.6N Sulfuric acid or TMB Stop Solution to the microwell plate. This will halt color development and will turn the SureBlue Reserve 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 hour.

### **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 readable.

## **ABSORBANCE MEASUREMENTS**

### **Kinetic Assays**

The SureBlue Reserve substrate produces a blue color upon reaction with peroxidase. Read at a wavelength of 620 - 650 nm.

### **Endpoint Assays**

The addition of 100  $\mu$ L (or an equal volume) of stop solution to the microwell plate will halt color development and will turn the SureBlue Reserve substrate yellow. Read at a wavelength of 450 nm. Stopped reactions should be read within 1 hour.

## **RECOMMENDED STOP SOLUTIONS**

For best results, KPL recommends 1N HCl, 0.6 N Sulfuric acid, or KPL's TMB Stop Solution (See Related Products) to stop the reaction.

## **PRODUCT SAFETY AND HANDLING**

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

## **RELATED PRODUCTS**

|   |                      |
|---|----------------------|
| 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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