KPL TECHNICAL SERVICE REPORT

Stability of SureBlue TMB 1-Component Microwell Peroxidase Substrate (Cat. No. 52-00-00, 52-00-01, 52-00-02, 52-00-03, 52-00-04, 52-00-05)

PURPOSE

To measure the stability and performance of the SureBlue TMB 1-Component Microwell Peroxidase Substrate when stored at 2 – 8 º over a three year period.

MATERIALS AND METHODS

The following lots of material were tested. Representative bottles from each lot were stored at 4ºC (recommended storage). Testing was completed in July 2009. The lot numbers are listed below:

Lot No.	Date of Manufacture
060212	03/01/06
060892	12/06/06
070244	02/21/07
070877	08/15/07
080481	04/16/08
081085	10/08/08
090595	06/04/09

The samples were evaluated via a standard EIA comparative assay. BSA-coated plates were treated with an HRP-conjugate dilution series. Different lots of SureBlue 1-Component TMB substrate were then added and allowed to react. The plates were stopped with TMB Stop Solution. Readings were taken at 12 minutes and compared to determine the percent variation that occurred between the different substrate lots. Background measurements of unreacted substrate were collected as well to ensure that the background had not changed over time.

The assay was performed as follows at room temperature:

1. 10% BSA Diluent Block was diluted 1:10 in Reagent Quality Water. 100 µL of this solution was added to each well of two clear Nunc MaxiSorp Medium Binding plates. They were allowed to incubate for 30 minutes. The solution was then discarded.
2. HRP conjugate was diluted to a concentration of 10 ng/mL in 1% BSA Diluent/Blocking solution. The conjugate was diluted 1:1.5 seven times, to a final concentration of 0.88 ng/mL.
3. 20 µL of each dilution was added to each row starting with A (10 ng/mL) and culminating with G (0.88 ng/mL). Row H contained 20 µL of 1% BSA Diluent/Block as a control.
4. 100 µL of SureBlue was added in triplicate to the requisite columns and allowed to react for 12 minutes. Lot Number 090595 was added to each plate to serve as a control.
5. 100 µL of TMB Stop Solution was then added to each well.
6. The plates were read at 450 nm.
7. The % Variation was calculated for each lot by comparing the slope of the reference sample to that of the other samples. This helps to demonstrate lot to lot consistency. The background was measured as well.

RESULTS

All lots of SureBlue TMB 1-Component Peroxidase Substrate are at or within 10% variation from one another, as demonstrated in Figure 1. Additionally, all lots demonstrate a background reading of 0.09 OD or less, as demonstrated in Figure 3.

Figure 1. Percent Variation between Different Lots of SureBlueTMB 1-Component Peroxidase Substrate.

Lot No.	Average Slope	% Variation
060212	0.1664	6.0
060892	0.1719	9.1
070244	0.1593	1.4
070877	0.1735	9.9
080481	0.1553	1.1
081085	0.1641	4.4
090595	0.157	N/A

Figure 2. Graphical Representation of the Performance of SureBlue TMB 1-Component Peroxidase Substrate

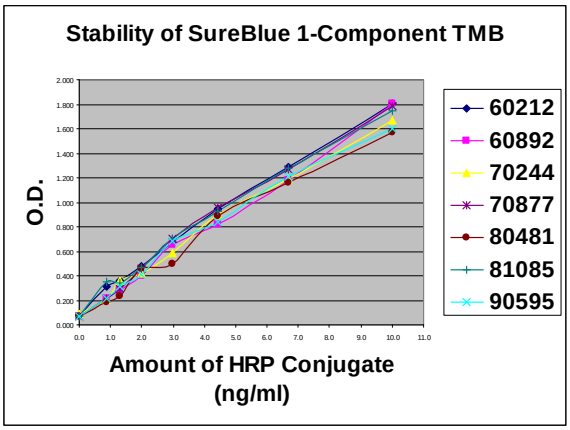

Figure 3. Background Readings for SureBlue TMB 1-Component Peroxidase Substrate.

Lot No.	Background (O.D.)
060212	0.074
060892	0.069
070244	0.088
070877	0.069
080481	0.068
081085	0.065
090595	0.072

CONCLUSIONS

KPL's SureBlue TMB 1-Component Peroxidase Substrate will maintain activity and background levels for a minimum of 3 years when stored at 2 – 8 ºC.

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