

# TECHNICAL SERVICE REPORT

## Stability of SureBlue Reserve™ TMB 1-Component Microwell Peroxidase Substrate (Catalog No. 53-00-01, 53-00-02, 53-00-03, 53-00-04)

### Purpose

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

### Materials and Methods

Three separate lots were tested. Representative bottles from each lot were stored at 4° C (recommended storage). Testing was completed May 2013. The lot numbers are listed below.

Lot No.	Date of Manufacture
100098	04/13/2010
100355	04/12/2010
100413	05/13/2010

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 Reserve 1-Component TMB substrate were then added and allowed to react. The plates were stopped with TMB Stop Solution. Readings were observed over a period of three years to assess performance as well as lot to lot variation. 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. 100 µL of TMB Stop Solution was then added to each well.

5. The plates were read at 450 nm.
6. Readings at three concentrations (6.7 ng/mL, 3.0 ng/mL and 1.3 ng/mL) were observed over time to look for trends.
7. The % Variation was calculated for each lot by comparing the stability lot to a freshly manufactured lot. This helps to demonstrate lot to lot consistency. The background was measured as well.

### Results

All lots of SureBlue Reserve TMB 1-Component Peroxidase Substrate are at or within 10% variation from one another. No appreciable loss in activity is observed over the time course (0 – 36 months). Additionally, all lots demonstrate a background reading of 0.09 OD or less.

Figure 1. Average Percent Variation between Different Lots of SureBlue Reserve TMB 1-Component Peroxidase Substrate.

Test Date	Average % Variation
07/12/2010	6.0
04/04/2011	2.8
10/05/2011	4.0
05/31/2012	4.5
12/04/2012	2.9
05/30/2013	5.7

Figure 2. 3.0 ng/mL HRP Conjugate Concentration

Month	Test Date	100098	100413	100355
3	07/12/2010	0.589	0.752	0.764
12	04/04/2011	0.814	0.783	0.824
18	10/05/2011	0.870	0.817	0.830
24	05/31/2012	0.799	0.740	0.717
30	12/04/2012	0.919	0.944	0.928
36	05/30/2013	0.660	0.671	0.662

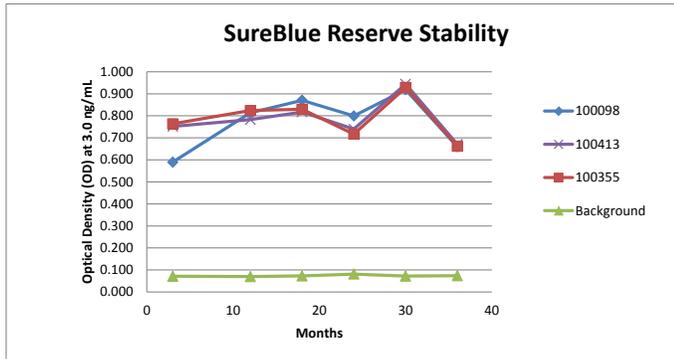


Figure 4. 6.7 ng/mL HRP Conjugate Concentration

Month	Test Date	100098	100413	100355
3	07/12/2010	1.588	1.387	1.875
12	04/04/2011	1.760	1.662	1.536
18	10/05/2011	1.735	1.667	1.647
24	05/31/2012	1.654	1.516	1.293
30	12/04/2012	1.960	1.939	2.039
36	05/30/2013	1.408	1.466	1.421

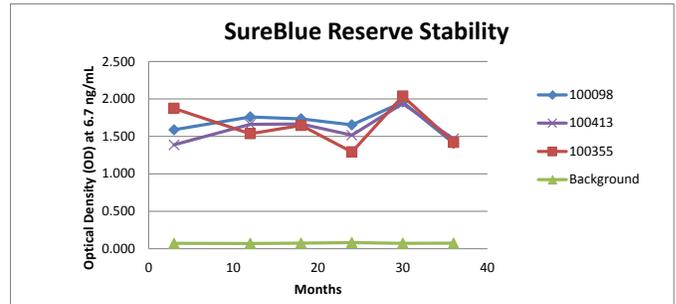
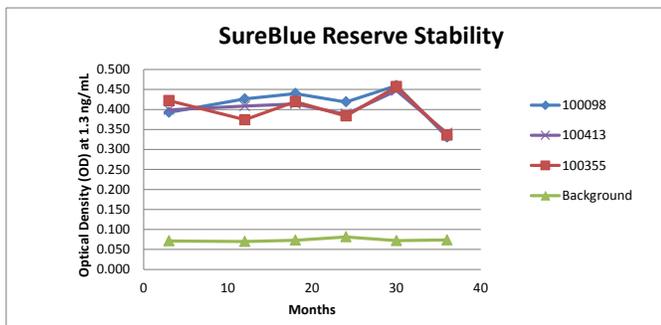


Figure 3. 1.3 ng/mL HRP Conjugate Concentration

Month	Test Date	100098	100413	100355
3	07/12/2010	0.393	0.399	0.422
12	04/04/2011	0.426	0.408	0.374
18	10/05/2011	0.440	0.414	0.420
24	05/31/2012	0.419	0.388	0.384
30	12/04/2012	0.460	0.447	0.457
36	05/30/2013	0.331	0.341	0.336



## Conclusion

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

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