KPL TECHNICAL SERVICE REPORT

Stability of TMB Stop Solution
(Cat. No. 50-85-04, 50-85-05, 50-85-06)

PURPOSE
To measure the stability and performance of the KPL TMB Stop Solution.

MATERIALS AND METHODS
The following lots of material were tested via a comparative assay. Representative bottles from each lot were stored at room temperature (recommended storage). Testing was completed in April 2009. The lot numbers are listed below:

Lot No.	Date of Manufacture
050104	04/25/05
050725	07/22/05
060655	09/18/06
070039	01/03/07
070415	04/09/07
081161	12/17/08

The samples were evaluated via a standard EIA comparative assay. BSA-coated plates were treated with an HRP-conjugate dilution series. SureBlue 1-Component TMB substrate was then added to each well. SureBlue 1-Component TMB Substrate was selected to represent our family of Microwell TMB substrates (TMB 2-Component Microwell Peroxidase Substrate System, SureBlue and SureBlue Reserve 1-Component TMB Substrates) due to its ease of use and similarity to our other two products when stopped. The plates were stopped with the aforementioned lots of TMB Stop Solution. Readings were taken at various time points and compared to determine the percent variation that occurred both between time points with individual lots, and between different lots of TMB Stop Solution.

EIA Reagents	KPL Lot No.
HRP, Goat Anti-Human IgG (H+L)	YM054
SureBlue TMB 1-Component	090122
10% BSA Diluent/Block Solution	080974

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 six times.
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 to each well and allowed to react for 12 minutes.
5. The TMB Stop Solutions were added in triplicate to their respective columns on each plate and allowed to react for 5 minutes. Lot No. 081161 was added to both plates to allow for a control between the two.
6. The plate was read after 5 minutes (Time Zero), then at 15, 30, 45 minutes and 1 hour.
7. The % Variation was calculated for each lot by comparing the average OD values at Time Zero and 60 minutes.

RESULTS

All lots of TMB Stop Solution are at or within 20% variation from Time Zero to 60 Minutes, as demonstrated in Figure 1.

Figure 1. Percent Variation between Time Zero and 60 minutes with Different Lots of TMB Stop Solution.

Lot No.	Average OD Maximum (Time Zero)	Average OD Minimum (60 Minutes)	% Variation
050104	0.807	0.645	20
050725	0.740	0.650	12.2
060655	0.778	0.656	15.1
070039	0.838	0.674	19.6
070415	0.837	0.720	14
081161	0.771	0.651	15.6

CONCLUSIONS

KPL's TMB Stop Solution will reliably stop KPL TMB microwell substrates for a minimum of 4 years after the date of manufacture.

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