ABTS Peroxidase Substrate Solution
Peroxidase Substrate Solution B
ABTS Peroxidase Substrate (1-Component)
TMB Peroxidase Substrate Solution

Technical Service Report

High-Density Polyethylene vs. Glass Bottles Stability Study

Purpose:

To compare the stability of ABTS Peroxidase Substrate Solution, Peroxidase Substrate Solution B, ABTS Peroxidase Substrate (1-Component), and TMB Peroxidase Substrate Solution in amber Nalgene high-density polyethylene (HDPE) and glass bottles over a one year period.

Reagents:

This study involved placing representative samples of the substrate solutions in both HDPE and glass (control) bottles. Samples were placed at 4°C, room temperature, and 37°C for 1 year. The following lots of substrate solutions were used:

Substrate Solution	Lot Number
ABTS Peroxidase Substrate	KD15
Peroxidase Substrate Solution B	KK04
ABTS Peroxidase Substrate (1-Component)	KD26
TMB Peroxidase Substrate	KE28

Test Parameters:

The substrate solutions were evaluated using an ELISA test procedure. The assays were performed on four REO virus-sensitized microtiter plates (Lot LH55) as follows:

1. Add 100 µl REO Positive Control Serum (Lot JG16) diluted 1/100 in Dilution Buffer (Lot KG77) to all wells in Rows A-G. To each well in Row H, add 100 µl of Normal Control Serum (Lot JH43) diluted 1/100 in Dilution Buffer. Incubate 30 minutes at room temperature.
2. Wash plates 3 times with Wash Solution Concentrate with 3 minute soak periods between washings.
3. Add 100 µl Peroxidase-labeled Goat anti-Chicken IgG (H+L) (Lot JM44-5), diluted 1/100 in Dilution Buffer to all wells. Incubate 30 minutes at room temperature.
4. Wash as in Step 2.
5. Add 100 µl of the appropriate substrate simultaneously to corresponding wells (Fig. 1).
   Note: Test samples of ABTS Peroxidase Substrate Solution and TMB Peroxidase Substrate Solution are mixed separately with an equal volume of Peroxidase Substrate Solution B (Lot KC79) that has been stored at 4°C. Test samples of Peroxidase Substrate Solution B are mixed with an equal volume of ABTS Peroxidase Substrate (Lot KM24).
6. After 15 minutes incubation at room temperature, the O.D. for each well is determined by the Dynatech MR650 ELISA reader at a wavelength of 410 nm for the plates testing the ABTS Peroxidase Substrate Solution, Peroxidase Substrate Solution B, and the ABTS Peroxidase Substrate (1-Component). The plate testing the TMB Peroxidase Substrate Solution is read at 650 nm.

Results:

In this study, samples of the four substrate solutions showed no significant change in average O.D. values as a result of storage in the HDPE bottles over a 1 year period . Samples stored at elevated temperatures (room temperature and 37°C) yielded results that on the average were no lower than samples stored in glass bottles. Storage at these elevated temperatures did result in a performance loss for the ABTS Peroxidase Substrate Solution and the ABTS Peroxidase Substrate (1-Component). This loss was consistant for samples stored in HDPE and glass bottles.

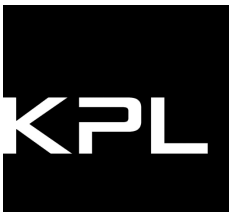
Conclusions:

The ABTS Peroxidase Substrate Solution, Peroxidase Substrate Solution B, ABTS Peroxidase Substrate (1-Component), and TMB Peroxidase Substrate Solution appear equally stable in high-density polyethylene bottles and glass bottles. Storage in the HDPE bottles seems to have no effect on product performance or sensitivity.

Figure 1. Illustration of plate layout showing O.D. data for Peroxidase Substrate Solution B

	Glass Bottles						HDPE Bottles					
	4°C		RT		37°C		4°C		RT		37°C	
	1	2	3	4	5	6	7	8	9	10	11	12
A	1.810	1.712	1.522	1.659	1.576	1.572	1.659	1.687	1.583	1.687	1.654	1.761
B	1.803	1.650	1.579	1.532	1.557	1.472	1.607	1.636	1.516	1.624	1.579	1.673
C	1.797	1.628	1.516	1.607	1.506	1.578	1.595	1.557	1.536	1.624	1.557	1.723
D	1.697	1.654	1.603	1.615	1.512	1.568	1.632	1.583	1.530	1.526	1.650	1.650
E	1.761	1.603	1.632	1.611	1.663	1.553	1.619	1.564	1.550	1.615	1.543	1.697
F	1.697	1.668	1.624	1.663	1.595	1.583	1.490	1.539	1.561	1.587	1.561	1.739
G	1.750	1.702	1.654	1.568	1.702	1.583	1.659	1.677	1.564	1.595	1.619	1.663
NCS	0.231	0.235	0.227	0.221	0.223	0.230	0.222	0.229	0.221	0.228	0.220	0.226
* Avg. O.D.	1.476		1.375		1.346		1.382		1.358		1.425	

*The O.D. value for the NCS well is substracted from the average of the O.D values of Rows A-G.



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