Technical Service Report

Stability of the ABTS Peroxidase Substrate System

Purpose:
To evaluate the stability of the 2 components of the ABTS Peroxidase Substrate System stored at 4°C and room temperature over time.

Reagents:
This study compares the performance of six lots of ABTS Peroxidase Substrate and six lots of Peroxidase Substrate Solution B. Representative samples of each of the following lots were stored at 4°C and room temperature from the date of manufacture.

ABTS Lot No.	Date of Mfg.	Sol. B Lot No.	Date of Mfg.
DF05	6/82	DF07	6/82
EC12	3/83	EH03	8/83
FD03	4/84	FD04	4/84
GC17	3/85	GC18	3/85
JE08	5/87	JC07	3/87
KD15	4/88	KC04	3/88

Test Parameters:
The components were evaluated using an ELISA test procedure. The assays were performed on two REO virus sensitized microtiter plates (Lot KH06). Plate 1 compared the performance of the different lots of the ABTS Peroxidase Substrate component and Plate 2 compared the lots of the Peroxidase Substrate Solution B component. The assay was performed 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 using Wash Solution Concentrate with 3 minute soak periods between washings.
3. Add 100 µl Peroxidase-labeled Goat anti-Chicken IgG (H+L) (Lot KH56), diluted 1/100 in Dilution Buffer to all wells. Incubate at room temperature for 30 minutes.
4. Wash as in Step 2.
5. Add 100 µl of the ABTS Peroxidase Substrate System simultaneously to appropriate wells (Fig. 1&2). In order to test each reagent of the 2-component system independently, the test lots for each component are mixed with a single recent lot of the other component.
   - On Plate 1 (Fig. 1), all test lots of ABTS Peroxidase Substrate are mixed with an equal volume of Peroxidase Substrate Solution B (Lot KC79).
   - On Plate 2 (Fig. 2), all test lots of Peroxidase Substrate Solution B are mixed with an equal volume of ABTS Peroxidase Substrate (Lot JC26).
6. After 30 minutes incubation at room temperature, the 0.D. for each well is determined by the Dynatech MR650 ELISA reader at a wavelength of 410 nm.

Results:
In this study, samples of ABTS Peroxidase Substrate and Peroxidase Substrate Solution B, when stored at 4°C show no significant variation in average O.D. between the lots (Figure 1-4). The ABTS Peroxidase Substrate samples stored at room temperature show a decrease in activity from an average O.D. value of 0.560 for the lot produced in 1988 to 0.245 for the lot produced in 1982 (Fig. 1). When stored at room temperature, Peroxidase Substrate Solution B has an average O.D. value range between 0.544-0.408 (Fig. 2). A slight decrease in activity over time is seen. The normal control serum values for plate 1, ABTS Peroxidase Substrate, indicate that the background color increases as a function of time. The samples stored at room temperature exhibit higher background O.D.'s than the corresponding 4°C samples. However, Peroxidase Substrate Solution B (Plate 2) shows no significant variation in background as a function of either time or temperature.

Conclusions:
KPL's ABTS Peroxidase Substrate System provides stable performance over a period of six years. Both components of the ABTS Peroxidase Substrate System appear very stable over time when stored at 4°C, the recommended storage temperature. Room temperature storage adversely affects the functionality of the components, the ABTS Peroxidase Substrate shows a substantial decrease in activity over time while the Peroxidase Substrate Solution B exhibits only a slight reduction. This study also demonstrates the exceptional consistency in product performance when stored at 4°C over an extended period of time.

Plate 1.
ABTS Peroxidase Substrate

	Lot DF05		Lot EC12		Lot FD03		Lot GC17		Lot JE08		Lot KD15	
	4°C	RT	4°C	RT	4°C	RT	4°C	RT	4°C	RT	4°C	RT
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.598	0.353	0.587	0.355	0.636	0.464	0.615	0.447	0.603	0.570	0.657	0.724
B	0.652	0.318	0.581	0.364	0.635	0.452	0.617	0.414	0.604	0.546	0.588	0.643
C	0.625	0.332	0.584	0.343	0.620	0.459	0.535	0.438	0.541	0.495	0.560	0.602
D	0.596	0.333	0.632	0.323	0.603	0.431	0.605	0.394	0.602	0.516	0.573	0.633
E	0.565	0.360	0.625	0.357	0.554	0.460	0.567	0.413	0.571	0.537	0.581	0.535
F	0.649	0.359	0.655	0.323	0.630	0.467	0.648	0.401	0.576	0.516	0.582	0.658
G	0.677	0.352	0.594	0.347	0.515	0.469	0.646	0.418	0.598	0.561	0.613	0.611
NCS	0.081	0.099	0.082	0.099	0.071	0.079	0.071	0.072	0.066	0.067	0.068	0.069
* Avg. O.D.	0.542	0.245	0.526	0.246	0.528	0.378	0.534	0.346	0.519	0.467	0.525	0.560

Figure 1.

Plate 2.
Peroxidase Substrate Solution B

	Lot DF07		Lot EH03		Lot FD04		Lot GC18		Lot JC07		Lot KC04	
	4°C	RT	4°C	RT	4°C	RT	4°C	RT	4°C	RT	4°C	RT
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.598	0.515	0.594	0.484	0.595	0.478	0.594	0.535	0.625	0.645	0.656	0.681
B	0.617	0.486	0.617	0.454	0.620	0.482	0.617	0.428	0.522	0.577	0.579	0.597
C	0.596	0.514	0.615	0.467	0.637	0.435	0.542	0.455	0.541	0.469	0.514	0.606
D	0.599	0.525	0.620	0.471	0.644	0.510	0.567	0.451	0.583	0.565	0.534	0.593
E	0.580	0.516	0.586	0.493	0.619	0.473	0.576	0.438	0.533	0.569	0.524	0.606
F	0.617	0.521	0.630	0.482	0.639	0.485	0.573	0.493	0.541	0.586	0.522	0.590
G	0.584	0.530	0.590	0.446	0.573	0.510	0.640	0.463	0.579	0.568	0.522	0.590
NCS	0.067	0.065	0.072	0.062	0.075	0.064	0.066	0.058	0.066	0.064	0.064	0.065
* Avg. O.D.	0.532	0.450	0.535	0.409	0.543	0.418	0.521	0.408	0.495	0.504	0.486	0.544

Figure 2.

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

Figure 3.

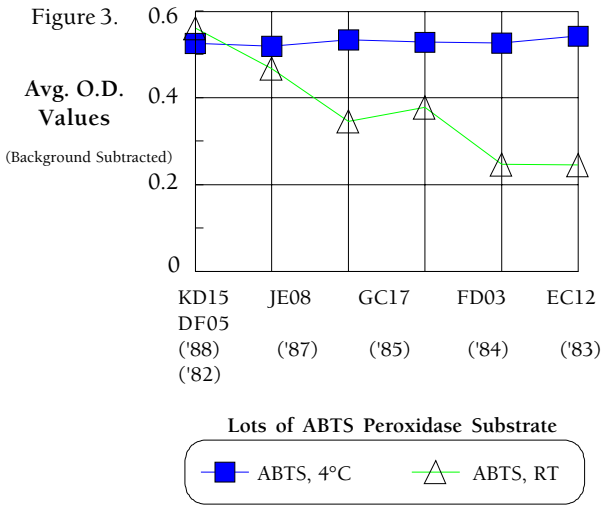
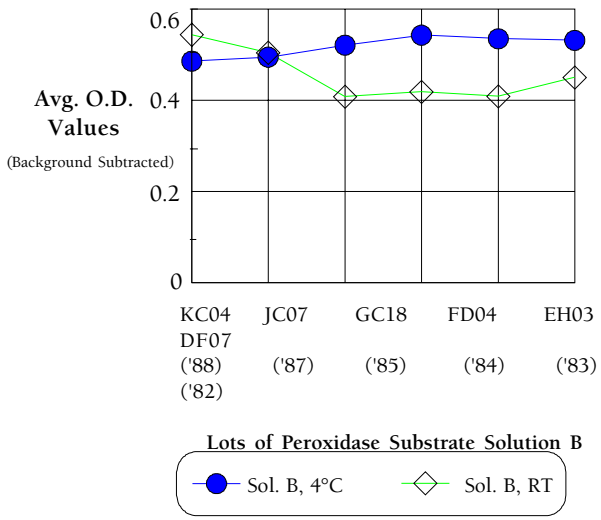


Figure 4.



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