Technical Service Report

Comparison of ABTS Peroxidase Substrate: One vs. Two Component Systems

Purpose:

To compare the performance of KPL One Component and Two Component ABTS Peroxidase Substrates.

Reagents:

Two lots of one component substrate were compared to two lots of ABTS Peroxidase Substrate and Peroxidase Solution B. All samples were QA retains that had been stored at 4°C since the date of manufacture.

Lot Numbers			
1 Component ABTS	2 Component ABTS	Peroxide Solution B	Year of Manufacture
NG49	ND10	NC51	1991
MF12	MD29	MF11	1990

Test Parameters: The substrates were assayed using a microwell ELISA procedure as follows:

1. Add 100 µl Human IgG (Cappel; Lot 34428) diluted to 1 µg/ml in PBS to all wells in Rows A-G. To each well in Row H add 100 µl of PBS. Incubate one hour at room temperature.
2. Prepare BSA Diluent/Blocking Solution Concentrate (Lot NE05) by diluting 1:10 in reagent quality water.
3. Add 300 µl of BSA Diluent/Blocking Solution to all wells. Incubate 5 minutes.
4. Prepare 10 ml of a 1:1000 dilution (0.5 µg/ml) of Peroxidase-Labeled Goat Anti-Human IgG (H+L), Lot NJ01-5, in BSA Diluent/ Blocking Solution.
5. Add 100 µl of the diluted conjugate to all wells in rows A-H. Incubate one hour at room temperature.
6. Wash plate 5 times with Wash Solution Concentrate (Lot ND25) using an automatic Skatron plate washer.
7. Add 100 µl of each substrate solution simultaneously to the appropriate wells (Figure 1) . Two component ABTS substrates were prepared by mixing equal volumes of ABTS Peroxidase Substrate with Peroxidase Substrate Solution B.
8. Read the O.D. for each well four minutes after adding substrate using the Dynatech MR650 ELISA reader with a 410 nm filter.

Results:

The average positive control O.D. for the two component substrate produced in 1991 was equal to the average O.D. of the corresponding one component system. For the substrate lots produced in 1990, the two component system had average positive values that were less than 4% lower than the average values of the one component substrate. The average O.D. of the negative control wells (Row H) for both the two component and the one component substrate systems was less than 0.072 O.D.

Conclusions:

The performance of KPL's two component ABTS Peroxidase Substrate system is equivalent to the performance of the one component substrate. The two products are equal in sensitivity and background color development. Both systems perform equally well after one year of storage at 4°C.

Figure 1.
ABTS
Peroxidase Substrate

	NG49			MF12			ND10/NC51			MD29/MF11		
	1	2	3	4	5	6	7	8	9	10	11	12
A	1.389	1.238	1.332	1.413	1.305	1.442	1.389	1.302	1.260	1.338	1.251	1.325
B	1.263	1.174	1.127	1.169	1.186	1.140	1.266	1.184	1.140	1.204	1.131	1.179
C	1.352	1.211	1.167	1.186	1.179	1.116	1.137	1.100	1.100	1.092	1.054	1.114
D	1.342	1.169	1.184	1.263	1.266	1.144	1.123	1.176	1.062	1.067	1.116	1.174
E	1.328	1.104	1.266	1.299	1.146	1.024	1.092	1.106	1.033	1.049	1.090	1.108
F	1.211	1.098	1.142	1.149	1.137	1.146	1.045	1.044	1.088	1.051	1.069	1.106
G	1.224	1.232	1.263	1.263	1.257	1.193	1.201	1.249	1.269	1.309	1.221	1.176
H	0.067	0.066	0.068	0.075	0.069	0.069	0.057	0.057	0.056	0.056	0.058	0.055
AVG OD-Row H	1.104			1.140			1.103			1.097		

ML-129-02

2 Cessna Court, Gaithersburg, MD 20879-4174 USA 301.948.7755 800.638.3167 FAX 301.948.0169