Stability of One Component ABTS Substrate

Purpose:

To evaluate the stability of One Component ABTS Peroxidase Substrate stored at 4°C and room temperature.

Reagents:

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

Lot Number	Date of Mfg.
MD23	4/90
NH15	7/91
PJ31	9/92
QF28	6/93
RK62	11/94
SA34	2/95

Test Parameters:

Substrates were assayed using a microwell ELISA procedure as follows:

1. Add 100 µl Human IgG (Cappel; Lot 38208) diluted to 1 µg/ml in PBS to all wells on a microwell ELISA plate. Incubate one hour at room temperature.
2. Prepare BSA Diluent/Blocking Solution Concentrate (Lot RK35) by diluting 1:10 in reagent quality water.
3. Add 300 µl of BSA Diluent/Blocking Solution to all wells. Incubate 15 minutes.
4. Prepare a 0.5 µg/ml dilution of Peroxidase-Labeled Goat Anti-Human IgG (H+L), Lot RK14, in BSA Diluent/Blocking Solution.
5. Add 200 µl of diluted conjugate to all test wells in row A. Add 100 ul of BSA Diluent/Blocking Solution to all test wells in rows B - H and titrate the conjugate serially down the plate through row G. Incubate for one hour at room temperature.
6. Wash plate 5 times with Wash Solution Concentrate (Lot RK37) using an automatic Skatron plate washer.
7. Add 100 µl of each substrate solution simultaneously to the appropriate wells (Figure 1) and incubate 15 minutes.
8. Read the O.D. for each well after 15 minutes using the Bio-Tek Microplate E1311 ELISA reader with a 405 nm filter.
9. Measure O.D. for each lot of substrate solution at 405 nm using a Perkin Elmer spectrophotometer (Figure 2).

Results:

Figure 1 shows that all samples of One Component ABTS Peroxidase Substrate are functional when tested by ELISA. O.D. values for Lot MD23, stored for over four years, are within 50% of O.D. values for Lot SA34, stored for only four months. Background color increases with longer storage time and is higher for samples stored at room temperature than for samples stored at 4°C. When inspected visually, all samples have a slight green or yellow tinge and are free of precipitate.

Conclusions:

KPL's One Component ABTS Peroxidase Substrate provides stable performance over a period of five years when stored at 4°C or room temperature.

Figure 1. ABTS Peroxidase Substrate after fifteen minutes

	MD23		NH15		PJ31		QF28		RK62		SA34	
	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	1.896	1.602	1.951	1.801	1.972	1.783	2.067	1.890	1.939	1.914	1.931	1.957
B	1.532	1.291	1.627	1.437	1.688	1.496	1.573	1.150	1.724	1.585	1.654	1.612
C	1.070	0.842	1.215	0.869	1.271	0.925	1.228	1.154	1.371	1.258	1.408	1.214
D	0.659	0.454	0.612	0.481	0.668	0.520	0.754	0.601	0.765	0.742	0.863	0.753
E	0.330	0.293	0.389	0.304	0.441	0.315	0.481	0.460	0.509	0.457	0.572	0.533
F	0.251	0.217	0.264	0.244	0.300	0.243	0.345	0.294	0.371	0.322	0.363	0.353
G	0.200	0.191	0.189	0.188	0.188	0.193	0.202	0.214	0.192	0.220	0.235	0.220
H	0.085	0.141	0.078	0.127	0.075	0.109	0.071	0.095	0.068	0.073	0.066	0.071

Figure 2. ABTS Peroxidase Substrate; absorbance at 405 nm.

	4°C	RT
MD23	0091	0.353
NH15	0.071	0.289
PJ31	0.059	0.228
QF28	0.042	0.148
RK62	0.043	0.125
SA34	0.020	0.041

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