



## Stability of BSA Diluent/Blocking Solution Concentrate

### Purpose:

To evaluate the performance of BSA Diluent/Blocking Solution Concentrate over time when stored at 4°C and room temperature.

### Reagents:

This study compares the performance of four lots of BSA Diluent/Blocking Solution. Representative samples of each of the following lots were stored at 4°C and room temperature respectively from the date of manufacture.

<u>Lot Number</u>	<u>Date of Mfg.</u>
HJ39	10/86
JK04	11/87
LD19	4/89
NE05	5/91

### Test Parameters:

The substrates were evaluated using a microwell ELISA test procedure. The assay was performed as follows:

1. Add 100 µl Human IgG (Cappel; Lot 34428) diluted to 10 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 working dilutions for each lot of BSA Diluent/Blocking Solution Concentrate by diluting each sample 1:10 in reagent quality water.
3. Add 300 µl of each BSA lot to all wells in the appropriate column on the microtiter plate. (See Fig. 1) Incubate 10 minutes.
4. Prepare 1 ml of a 1:100 dilution of Peroxidase-labeled Goat anti-Human IgG (H+L), Lot NA61-1, by adding 10 µl of conjugate to 1ml of BSA. Prepare conjugate in a separate tube for each lot of BSA Diluent/Blocking Solution.
5. Add 200 µl of the diluted conjugates to the appropriate wells in Row A. Add 100 µl of the corresponding BSA lot to all wells in rows B-H. Perform serial two-fold dilutions of the conjugate by transferring 100 µl from each well in Row A to the well below. Continue to make dilutions through Row H.
6. Incubate for one hour at room temperature.
7. Wash plate 5 times with Wash Solution Concentrate (Lot NA90) using an automatic Skatron plate washer.
8. Prepare ABTS Substrate Solution by mixing equal volumes of ABTS Substrate Solution (Lot NB07) and Substrate Solution B (Lot MF11).
9. Add 100 µl of substrate solution to all wells.
10. The O.D. for each well is determined by the Dynatech MR650 ELISA reader at 410 nm.

### Results:

Samples of BSA Diluent/Blocking Solution stored at either 4°C or room temperature show no significant variation in O.D. values among the test lots (Figure 1). The O.D. values for the BSA control wells (Row H) were comparable for all test samples, which indicates that storage of this product over time does not produce higher background.

### Conclusions:

KPL's BSA Diluent/Blocking Solution appears very stable over the five year study period. The performance of the product appears to be equivalent when stored at either 4°C or room temperature.

FIGURE 1.

	Lot HJ39			Lot JK04			Lot LD19			Lot NE05		
	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	1.354	1.408	1.416	1.354	1.345	1.371	1.470	1.466	1.438	1.454	1.427	1.405
B	0.989	1.073	1.085	0.977	1.030	1.030	1.237	1.186	1.211	1.223	1.165	1.180
C	0.680	0.716	0.703	0.652	0.672	0.685	0.910	0.895	0.903	0.899	0.833	0.832
D	0.383	0.416	0.501	0.381	0.391	0.406	0.550	0.563	0.575	0.568	0.490	0.487
E	0.236	0.244	0.295	0.219	0.230	0.237	0.312	0.320	0.328	0.314	0.277	0.281
F	0.152	0.155	0.181	0.146	0.141	0.151	0.190	0.194	0.192	0.201	0.194	0.187
G	0.108	0.112	0.123	0.101	0.104	0.109	0.123	0.125	0.125	0.136	0.128	0.129
H	0.064	0.064	0.061	0.059	0.059	0.065	0.059	0.057	0.056	0.057	0.060	0.063

