Stability of Milk Diluent/Blocking Solution Concentrate

Purpose:

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

Reagents:

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

Milk Diluent/Blocking Solution Lot No.	Date of Mfg.
HG16	7/86
JE25	5/87
KF10	6/88
LC25	3/89
MF49	7/90

Test Parameters:

The components were evaluated using an microwell ELISA test procedure. The assay was performed as follows:

1. Add 100 µl Goat anti-Salmonella (CSA-1), Lot MM42, at a concentration of 10 µg/ml in a 0.1M PBS buffer to all wells except columns 1&12 (See Figure 1). Incubate 1 hour at room temperature.
2. Block plate with 300 µl of a 1:20 dilution of Milk Diluent/Blocking Solution. Incubate for 30 minutes at room temperature. Note: The 4°C and room temperature samples from each lot of Milk Diluent/Blocking Solution are added to a column of the plate (Figure 1.).
3. Add 200 µl *Salmonella typhimurium* Positive Control (Lot GF05), starting at a 1/250 dilution in the respective Milk Diluent/Blocking Solutions to Row A. 150 µl of the respective test lots of 1X Milk Diluent/Blocking Solution are added to the remaining wells and serial 1:4 dilutions of the *S. typhimurium* are performed down the plate (i.e. Row B has a 1/1000 dilution, Row C 1:4000, etc.). Row H serves as a control and only the Milk Diluent/Blocking Solution is added to this row. Incubate at room temperature for 1 hour.
4. Wash plate 3 times with Wash Solution Concentrate with 3 minute soak periods between washings.
5. Add 100 µl of Peroxidase-labeled Goat anti-Salmonella (CSA-1), lot MA17, at a concentration of 1 µg/ml to all test wells. Again, the respective lot of Milk Diluent/Blocking Solution is used to dilute the peroxidase conjugate. Incubate at room temperature for 1 hour.
6. Wash plate as in step 4.
7. Add 100 µl of the ABTS Peroxidase Substrate System to all wells (Fig. 1). Equal volumes of the ABTS Peroxidase Substrate Solution, lot MH61, and Peroxidase Substrate Solution B, lot MK55, are combined to make the working substrate.
8. After 4 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.

Results:

In this study, samples of Milk Diluent/Blocking Solution, when stored at either 4°C or room temperature, show no significant variation in O.D. values between the lots (Figure 1). All samples stored at 4°C retained a milky opalescent color. The samples stored at room temperature appear amber in color. The degree of amber color increases with the time of storage at room temperature. The presence of variations in color does not seem to affect product performance.

Conclusions:

KPL's Milk Diluent/Blocking Solution appears very stable over a four year period when stored at 4°C or room temperature. Prolonged storage at room temperature results in amber color formation in the product which does not appear to affect peformance or background. When stored at 4°C, the recommended storage condition, no amber color can be seen. This study demonstrates the exceptional consistency in product performance over an extended period of time.

Figure 1.
ELISA plate O.D. values for the Milk Diluent/Blocking Solution stability assay.

		Lot HG16		Lot JE25		Lot KF10		Lot LC25		Lot MF49		
		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		1.387	1.512	1.325	1.432	1.196	1.307	1.366	1.468	1.363	1.337	
B		0.799	0.915	0.780	0.899	0.815	0.800	0.823	0.885	0.830	0.760	
C		0.432	0.448	0.424	0.461	0.431	0.394	0.360	0.443	0.375	0.373	
D		0.182	0.203	0.181	0.212	0.194	0.178	0.167	0.182	0.159	0.164	
E		0.099	0.112	0.105	0.118	0.107	0.098	0.091	0.099	0.094	0.098	
F		0.081	0.093	0.082	0.092	0.087	0.079	0.075	0.078	0.076	0.077	
G		0.077	0.089	0.079	0.089	0.087	0.074	0.071	0.073	0.073	0.074	
H		0.072	0.088	0.075	0.091	0.078	0.074	0.070	0.074	0.070	0.078	

S. typhimurium dilution:
1:250
1:1000
1:4000
1:16K
1:64K
1:256K
1:1024K
Milk Dil/Block only

ML-119-02

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