KPL TECHNICAL SERVICE REPORT

Stability of TMB Substrate Solution

(Catalog No. 37-00-78, 50-76-01, 50-76-02, 50-76-08, 50-76-09)

PURPOSE

The purpose is to evaluate the stability of the TMB Peroxidase Substrate (KPL Catalog Nos. 37-00-78, 50-76-01, 50-76-02, 50-76-08, 50-76-09) stored at 4°C and room temperature.

REAGENTS

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

TMB Lot No.	Date of Mfg.
ML24	11/90
NL42	12/91
PE16	4/92
QM07	11/93
RH32	8/94
SD86	5/95

PROCEDURES

The 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 (see Figure 1). Incubate one hour at room temperature.
2. Prepare BSA Diluent/Blocking Solution by diluting BSA Diluent/Blocking Solution Concentrate (KPL, Inc. Lot RK35) 1:10 in reagent quality water.
3. Empty the plate after one hour and add 300 µl of BSA Diluent/Blocking Solution to all wells. Incubate for fifteen minutes at room temperature.
4. Dilute Peroxidase-Labeled Goat Anti-Human IgG (H+L), KPL, Inc. Lot RK14, to 0.5 µg/mL 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. After one hour, empty the plate and wash 5 times with Wash Solution Concentrate (KPL, Inc. Lot RK37) using an automatic Skatron plate washer.
7. Mix each sample of TMB Substrate Solution with an equal volume of Peroxidase Substrate Solution B, Lot SD68.
8. Add 100 µL of each substrate solution to the appropriate wells (Figure 1) and incubate for 7 minutes.
9. Determine the O.D. for each well using the Bio-Tek Microplate EL311 ELISA reader with a 630 nm filter.
10. Measure O.D. for each lot of substrate solution at 650 nm using a Perkin Elmer spectrophotometer (Figure 2).

RESULTS

Figure 1 shows that sensitivity of TMB Microwell Substrate decreases with increasing storage time, with a greater loss of sensitivity for samples stored at room temperature than for samples stored at 4°C. O.D. values for Lot ML24, stored for over four years at either 4°C or room temperature, are within at least 50% of the O.D. values provided by lot SD86, which was stored for only one month. Background absorbance for all samples is 0.005 O.D. or less. Visually, all samples stored at 4°C were clear and colorless. Samples of Lots RH32 and SD86 stored at room temperature were also clear; other samples stored at room temperature had a yellow tinge.

Figure 1. ELISA data after 7 minutes (OD values at absorbance of 630nm).

	Lot ML24		Lot NL42		Lot PE16		Lot QM07		Lot RH32		Lot SD86	
	4°C	**RT**	**4°C**	**RT**	**4°C**	**RT**	**4°C**	**RT**	**4°C**	**RT**	**4°C**	**RT**
A	1.043	0.783	0.963	0.862	1.087	0.933	1.094	0.971	1.268	1.165	1.312	1.088
B	0.969	0.639	0.895	0.800	0.925	0.852	0.965	0.841	1.013	0.913	1.234	0.997
C	0.735	0.471	0.743	0.710	0.766	0.703	0.772	0.712	0.797	0.709	0.934	0.784
D	0.641	0.429	0.553	0.588	0.603	0.572	0.543	0.541	0.610	0.532	0.807	0.636
E	0.474	0.280	0.353	0.350	0.405	0.245	0.380	0.333	0.407	0.295	0.560	0.464
F	0.297	0.179	0.255	0.205	0.244	0.166	0.221	0.207	0.237	0.200	0.350	0.293
G	0.174	0.156	0.144	0.157	0.132	0.131	1.141	0.132	0.175	0.144	0.227	0.203
Blank	0.046	0.050	0.048	0.051	0.044	0.052	0.047	0.046	0.047	0.045	0.045	0.047

Figure 2. Background absorbance (630 nm) for various lots of TMB Peroxidase Substrate.

	4°C	RT
ML24	0.003	0.005
NL42	0.004	0.005
PE16	0.003	0.004
QM07	0.004	0.004
RH32	0.004	0.003
SD86	0.003	0.003

CONCLUSIONS

KPL's TMB Peroxidase Substrate maintains stable performance when stored at either 4°C or room temperature for up to four years. The long term stability of the Peroxidase Substrate Solution B, the second component in the TMB Microwell Substrate System, is demonstrated in the Stability Study of the Peroxidase Substrate Solution B (ML-114).

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