TMB Membrane Peroxidase Substrate
One Component

Technical Service Report

Stability of One Component TMB Membrane Peroxidase Substrate

Purpose:

To evaluate the stability of the KPL One Component TMB Membrane Peroxidase Substrate.

Reagents:

Samples of one component TMB Membrane Peroxidase Substrate were stored at 4°C and room temperature from the date of manufacture.

Lot No.	Date of Mfg.
NE33	5/91
PF27	5/92
QE19	4/93
RD71	4/94
SD65	5/95

Test Parameters:

The substrates were evaluated using a dot ELISA test procedure. The assays were performed on standard nitrocellulose membrane (Schleicher & Schuell) as follows:

1. Prepare two-fold dilutions of Human IgG (Cappel Lot 35712) in a microwell ELISA plate, starting at a concentration of 0.05 mg/ml in PBS.
2. Mark the nitrocellulose membrane by making a grid (Figure 1.), using an appropriate pen.
3. Wet the membrane with reagent quality water and blot off excess moisture.
4. Transfer 1.0 µl of the diluted Human IgG from each well in the dilution plate to the appropriate spot on duplicate gridded membrane strips using a microdispenser. Air dry strips for approximately 5 minutes to allow protein to adhere to the membrane.
5. Block strips with 0.5% Milk Diluent/Blocking Solution (Cat. No. 50-82-01), Lot QM14, for 15 minutes at room temperature.
6. Incubate strips with Peroxidase-Labeled Goat Anti-Human IgG (g) (Catalog No. 14-10-02), Lot PB45-5, diluted 1:10,000 in 0.5% Milk Diluent/Blocking Solution, for one hour at room temperature.
7. Wash strips two times with a 10 minute soak period for each wash using Wash Solution Concentrate (Cat. No. 50-63-00), Lot PA05. Rinse strips with water after washing.
8. Place strips in the appropriate TMB substrate lot. Incubate at room temperature for five minutes.
9. Stop substrate reaction after 5 minutes by rinsing the membranes in water for 10-20 seconds.
10. Allow strips to air dry and store sealed under plastic in the dark.

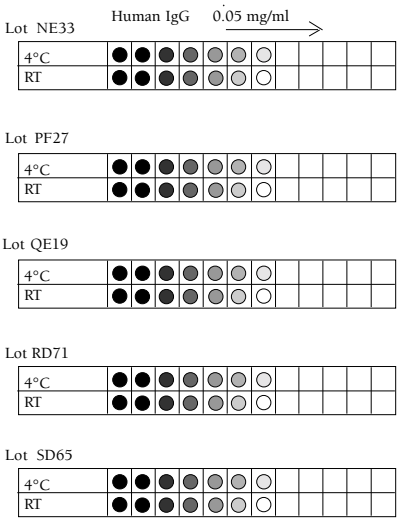
Results:

Human IgG was detected to an endpoint titer of 780 ng/ml. Samples stored at room temperature developed with slightly less color intensity at the endpoint than samples stored at 4°C. All lots produced equivalent color intensity and clear white background. In solution, all samples appeared clear and colorless to light brown, and were free of precipitate.

Conclusions:

KPL's One Component TMB Membrane Peroxidase Substrate demonstrates excellent stability when stored at either 4°C or room temperature for up to four years. Storage at 4°C is recommended for best results.

Figure 1.



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