Technical Service Report

Comparison of TMB Membrane Peroxidase Substrate: One vs. Three Component Systems

Purpose:

To compare the performance of KPL one component and three component TMB Membrane Peroxidase Substrate systems.

Reagents:

System	Lot Number	Unit Size	Cost	Price per ml
KPL(3 comp)	NH03/NH47/MH56	440 ml kit	$70.00	$0.16
KPL(1 comp)	NK34	2 x 100 ml	$50.00	$0.25

Test Parameters:

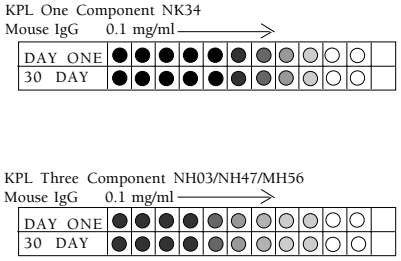
The test samples are evaluated using a dot ELISA test procedure. The assays are performed on standard nitrocellulose membrane (Schleicher & Schuell) as follows:

1. Set up dilution plate by performing 11 two-fold dilutions across a single row of a microtiter plate with Mouse IgG (Cappel Lot 34819), starting at a concentration of 0.1mg/ml in PBS.
2. Using an appropriate pen, mark the nitrocellulose membrane by making a grid (Figure 1.).
3. Wet the membrane with reagent quality water.
4. From each well in the dilution plate, transfer 1.0 µl of the diluted Mouse IgG to appropriate spot on duplicate gridded membrane strips using a microdispenser. Incubate strips for approximately 5 minutes to allow protein to adhere to the membrane.
5. Block strips with 0.2% Milk Diluent/Blocking Solution (Cat. No. 50-82-01) for 1 hour at room temperature.
6. Incubate strips with Peroxidase-Labeled Goat Anti-Mouse IgG (H+L), Catalog No. 04-18-06 (Lot ML13-1), diluted 1:500 in 0.1% Milk Diluent/Blocking Solution, for 30 minutes at room temperature.
7. Wash strips with a 45 minute soak period using Wash Solution Concentrate (Cat. No. 50-63-00). Rinse strips with water after washing.
8. To prepare KPL three-component substrate working solution, mix five parts TMB Peroxidase Substrate Solution, Product Code 50-76-01 (Lot NH03) with five parts Peroxidase Substrate Solution B, Product Code 50-65-00 (Lot NH47), and one part TMB Membrane Enhancer, Product Code 50-77-01 (Lot MH56).
9. Place strips in the appropriate TMB substrate and incubate at room temperature.
10. Stop substrate reaction after 4 minutes by rinsing the membranes in water for 10-20 seconds.
11. Allow strips to air dry and store sealed under plastic in the dark.

Results:

Both KPL systems appear to be equivalent in sensitivity, as they detected Mouse IgG to the same endpoint concentration (0.1 µg/ml). The one component system gave the most intense color development. After drying, the background color of the nitrocellulose paper was very clear for both substrate systems.

Figure 1.



Conclusions:

KPL's one component TMB Membrane Peroxidase Substrate appears to be equivalent in performance to the KPL three component system. No background is seen when the Milk Diluent/Blocking Solution is used.

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