Comparison of KPL Peroxidase Membrane Substrates

Purpose:

To compare the sensitivity of 4CN, DAB, and TMB Peroxidase Substrates on nitrocellulose membranes.

Reagents:

Substrate Components	Product Code	Lot Number	Date of Manufacture
TMB One Component	52-00-01	NL15	11/91
TMB Solution	50-76-01	NH03	8/91
Solution B	50-65-00	NH47	8/91
TMB Membrane Enhancer	50-77-01	NM49	12/91
4CN	50-73-05	MK01	10/90
4CN	50-73-05	MK79	10/90
DAB	71-00-46	NF30	6/91
DAB	71-00-46	NL34	11/91
Tris Buffer	71-00-47	NL35	11/91
Peroxide Solution	71-00-48	NL36	11/91

Procedure:

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

1. Prepare two-fold dilutions of Human IgG (Cappel Lot 34428) in a microwell ELISA plate, starting at a concentration of 0.1 mg/ml in PBS.
2. Mark the nitrocellulose membrane with a grid (Figure 1), using an appropriate pen.
3. Wet the membrane with reagent quality water.
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 tothe mem brane.
5. Block strips with 0.5% Milk Diluent/Blocking Solution (Product Code 50-82-00), Lot NJ03, for 15 minutes at room temperature.
6. Incubate strips with Peroxidase-Labeled Goat Anti-Human IgG (H+L), (Catalog No. 14-10-06), Lot NJ21-5, diluted 1:5000 in 0.5% Milk Diluent/Blocking Solution, for 1 hour at room temperature.
7. Wash strips with a 15 minute soak period using Wash Solution Concentrate (Product Code 50-63-02), Lot ND20, diluted 1:20 in reagent quality water. Rinse strips with reagent quality water after washing.
8. Prepare substrate working solutions as follows:

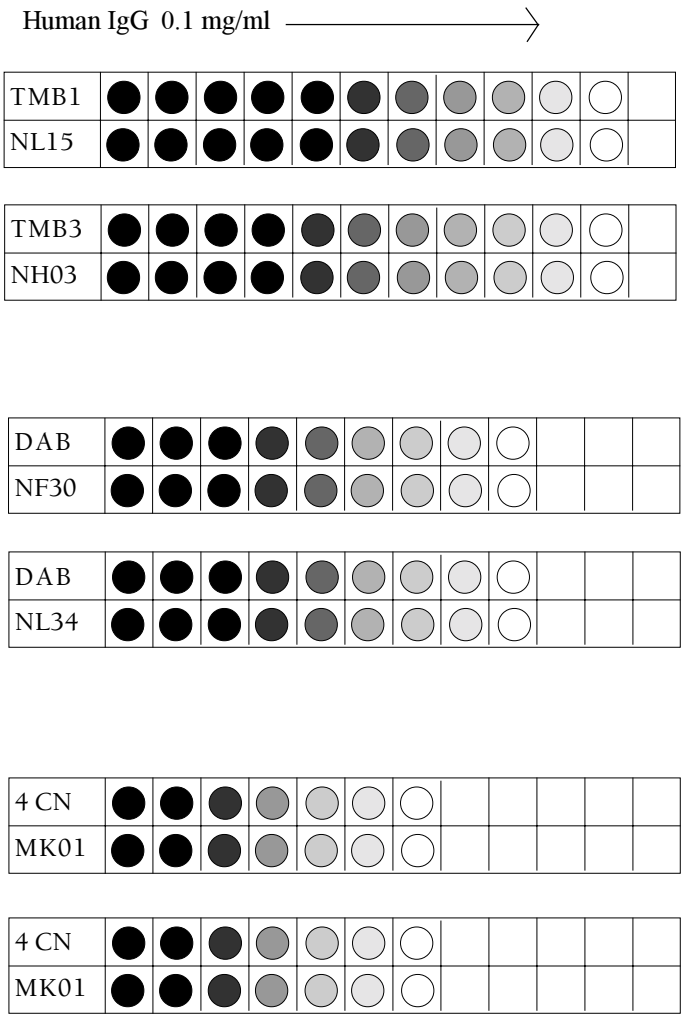
TMB 3 Component: Mix five parts TMB Peroxidase Substrate Solution (Lot NH03), five parts Peroxidase Substrate Solution B (Lot NH47), and one part TMB Membrane Enhancer (Lot NM49).

4CN: Mix equal volumes of 4CN (Lot MK01 or MK79) and Solution B (Lot NH47).

DAB Reagent Set: To 5 ml of reagent quality water, add 2 drops DAB (Lot NF30 or NL34), 3 drops Tris Buffer (Lot NL35), and 2 drops Peroxide Solution (Lot NL36).

9. Place strips in the appropriate substrate solution. Incubate at room temperature for 4 minutes.
10. Stop substrate reaction after 4 minutes by rinsing the strips in water for 10-20 seconds.
11. Allow strips to air dry. Store sealed under plastic in the dark.

Figure 1:



Results:

Human IgG was detected to the same endpoint concentration (0.1 µg/ml) for both TMB substrate systems, with greater intensity of color development observed for the one component system. Both lots of DAB substrate were equal in sensitivity (0.4 µg/ml) and intensity. 4CN is the least sensitive substrate, detecting antigen to an endpoint concentration of 1.5 µg/ml and producing a fainter color than the other substrates. Three component TMB turned the nitrocellulose paper slightly yellow after drying, while strips developed with all the other substrates exhibited minimal background staining.

Conclusions:

KPL One Component TMB Substrate is the most sensitive product, followed by the 3 component TMB system, DAB Reagent Set, and 4CN in descending order of sensitivity. All substrates are very consistent in performance.

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