



Comparison of KPL Peroxidase Membrane Substrates

Purpose:

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

Reagents:

<u>Substrate Components</u>		<u>Product Code</u>	<u>Lot Number</u>	<u>Date of Manufacture</u>
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 membrane (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 μ l 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 (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:

Human IgG 0.1 mg/ml \longrightarrow

TMB1	●	●	●	●	●	●	●	●	●	○	○	
NL15	●	●	●	●	●	●	●	●	●	○	○	

TMB3	●	●	●	●	●	●	●	●	●	○	○	
NH03	●	●	●	●	●	●	●	●	●	○	○	

DAB	●	●	●	●	●	●	●	○				
NF30	●	●	●	●	●	●	●	○				

DAB	●	●	●	●	●	●	●	○				
NL34	●	●	●	●	●	●	●	○				

4 CN	●	●	●	●	○	○	○					
MK01	●	●	●	●	○	○	○					

4 CN	●	●	●	●	○	○	○					
MK01	●	●	●	●	○	○	○					

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.

