

### Technical Service Report

# Sensitivity of TrueBlue Peroxidase Substrate on Membranes

## Purpose:

Reagents:

To evaluate the sensitivity of TrueBlue Peroxidase Substrate on membranes, compared to one component TMB Membrane Substrate, HistoMark BLACK, and DAB peroxidase substrates.

 Substrate	Lot Number		
TrueBlue Peroxidase Substrate	121693 (R&D)		
One Component TMB Membrane Peroxidase Substrate	RA38		
DAB Peroxidase Substrate	QA17		
HistoMark BLACK Peroxidase Substrate	OF10		

#### Test Parameters:

The substrates were evaluated using a dot ELISA test procedure on PVDF membrane (Millipore) as follows:

- 1. Prepare two-fold dilutions in PBS of Salmonella positive control (Product Code 50-91-99), beginning at 1:200.
- 2. Wet PVDF membrane strips with 100% methanol and blot off excess moisture.

Substrate

Peroxidase Solution

- 3. Apply 1.0 µl of each dilution of Positive Control to the appropriate spot on the membrane strips (Figure 1) using a microdispenser. Air dry strips for approximately 5 minutes to allow protein to adhere to the membrane.
- 4. Block strips with Milk Diluent/Blocking Buffer (Product Code 50-82-01), diluted 1:4 with reagent quality water, for 10 minutes at room temperature.
- 5. Incubate strips with Peroxidase-Labeled Goat Anti-Salmonella (Catalog No. 04-91-99) diluted to  $0.1~\mu g/ml$  in diluted Milk Diluent/Blocking Buffer, for four minutes at room temperature.
- 6. Wash strips 10 minutes in Wash Solution Concentrate (Cat. No. 50-63-00), diluted 1:20 in reagent quality water.
- 7. Prepare DAB substrate as follows:
  - Add 100 µl DAB substrate and 100 µl Peroxidase Solution to 5 ml reagent quality water and mix thoroughly.

Prepare HistoMark BLACK substrate as follows:

Add 5 ml Enhance BLACK Buffer and 100 µl DAB substrate to 5 ml reagent quality water and mix thoroughly. Add 100 µl Peroxidase Solution and mix thoroughly.

TMB substrates are ready to use.

- 8. Place strips in the appropriate substrate lot. Incubate at room temperature for 10 minutes.
- 9. Stop substrate reaction after one minute by rinsing the membranes in water for 20-30 seconds.
- 10. Allow strips to air dry and store sealed under plastic in the dark.

## Results:

 $Salmonella\ Positive\ Control\ was\ detected\ to\ an\ endpoint\ titer\ of\ 1:800\ with\ DAB;\ 1:3200\ with\ HistoMark\ BLACK;\ 1:12,800\ with\ TrueBlue\ Peroxidase\ Substrate;\ and\ 1:25,600\ with\ one\ component\ TMB\ Membrane\ Peroxidase\ Substrate.$ 

## Conclusions:

 $True Blue \ Peroxidase \ Substrate is approximately \ 4 times as sensitive \ as \ HistoMark \ BLACK \ and \ 16 times \ as sensitive \ as \ DAB \ when tested \ on \ membranes. \ One \ Component \ TMB \ Peroxidase \ Substrate \ has the highest sensitivity \ on \ membranes.$ 

Figure 1.

Salmonella Pos. Control diluted 2-fold from 1:200

Lat Number

QE44

DAB		0	0									
BLACK	•			0	0							
TrueBlue	•	•	•			0	0					
TMB	•	•	•	•			0	0				