Technical Service Report

Sensitivity of TrueBlue Peroxidase Substrate on Membranes

Purpose:

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

Reagents:

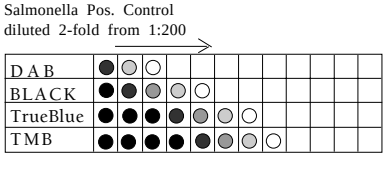
Substrate	Lot Number
TrueBlue Peroxidase Substrate	121693 (R&D)
One Component TMB Membrane Peroxidase Substrate	RA38
DAB Peroxidase Substrate	QA17
HistoMark BLACK Peroxidase Substrate	QF10
Peroxidase Solution	QE44

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.
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 µg/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.

Figure 1.



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:

TrueBlue 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.

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