Technical Service Report

Comparison of ABTS, TMB, and OPD Peroxidase Substrate Systems

Purpose:

To directly compare the sensitivity of the peroxidase substrates ABTS, TMB, and OPD and to compare the substrates stopped versus nonstopped.

Reagents:

The following peroxidase substrates and stop solutions were used:

Substrate Preparation and Composition	Stop Solution
- One Component TMB Peroxidase Substrate Solution Lot SD67	- KPL One Component TMB Stop Solution Lot SE53
- One Component ABTS Peroxidase Substrate Solution Lot SA34	- ABTS Peroxidase Stop Solution (1% SDS) Lot NE21
- One 30 mg OPD tablet Lot 065H-8953 (Sigma) + 30 ml Peroxidase Solution B (H_2O_2) Lot RJ34	- 3 M Sulfuric Acid

Test Parameters:

The substrates were assayed using a microwell ELISA procedure as follows:

1. Add 100 µl Human IgG (Cappel; Lot 35712) diluted to 100 ng/ml in PBS to all wells in columns 1-12 on a microwell ELISA plate. Incubate one hour at room temperature.
2. Prepare BSA Diluent/Blocking Solution by diluting BSA Diluent/Blocking Solution Concentrate (Lot SC08) 1:10 in reagent quality water.
3. Empty the plate after one hour and add 300 µl of BSA Diluent/Blocking Solution to all wells. Incubate for fifteen minutes at room temperature.
4. Dilute Peroxidase-Labeled Goat Anti-Human IgG (H+L), Lot LL32-1 to 1 µg/ml, in BSA Diluent/Blocking Solution.
5. Add 200 µl of the diluted conjugate to all test wells in row A. Add 100 µl of BSA Diluent/Blocking Solution to all test wells in rows B - H and titrate the conjugate serially down the plate through row G. Incubate for one hour at room temperature.
6. Wash plate 5 times with Wash Solution Concentrate (Lot PM08).
7. Add 100 µl of each substrate solution to two columns on the microtiter plate and incubate eight minutes.
8. After each substrate has incubated for eight minutes, add 100 µl of the appropriate stop solution.
9. Determine the O.D. for each well before and after stopping on the Bio-tek CERES 900HDi ELISA reader using the following filters as appropriate:
   - ABTS: 410 nm non-stopped and stopped
   - TMB: 620 nm non-stopped and 450 nm stopped
   - OPD: 450 nm non-stopped and 490 stopped

Results:
Before addition of stop solution TMB substrate produces the highest O.D. values of the substrates tested. ABTS and OPD substrates are approximately equivalent to each other and yield lower O.D. values than TMB. The addition of the appropriate stop solution increases the O.D. of TMB and OPD substrates by more than twice their corresponding non-stopped values. Upon stopping, TMB changes in color from blue to yellow, while OPD changes from yellow to orange. O. D. readings are higher for TMB substrate than for OPD after stopping. ABTS substrate remains green in color after addition of stop solution.

Figure 1: Substrate Performance Before Reaction is Stopped

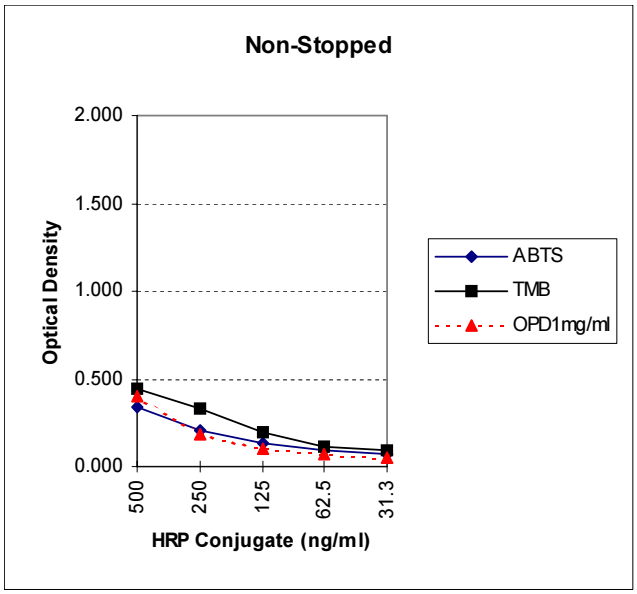
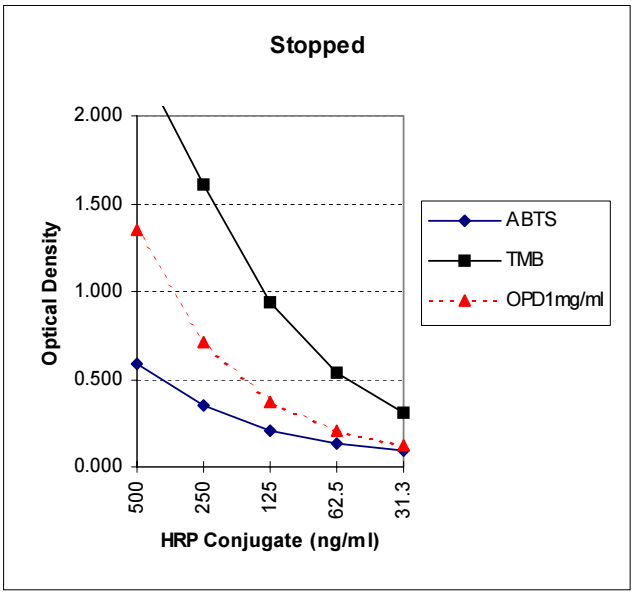


Figure 2: Substrate Performance After Reaction is Stopped



Conclusions:
This study suggests that the peroxidase substrate TMB is the most sensitive substrate and will yield the highest signal after the addition of stop solution. OPD is less sensitive than TMB, but still gives a higher signal than ABTS after the addition of stop solution. TMB and OPD both require the use of different filters for reading the respective stopped and nonstopped O.D. values, whereas ABTS requires the use of only one filter. Furthermore, the shift in O.D. values seen when stopping TMB and OPD demands closer monitoring of nonstopped substrate reactions in order to obtain readable O.D. values when the reactions are stopped. Unlike OPD, neither ABTS or TMB is considered a hazardous material.

ML-108-03

2 Cessna Court, Gaithersburg, MD 20879-4174 USA 301.948.7755 800.638.3167 FAX 301.948.0169