

Assay Development: Testing Reagents for Cross Reactivity

• Simple, fast method • Minimize reagent interactions • Reduce noise and background

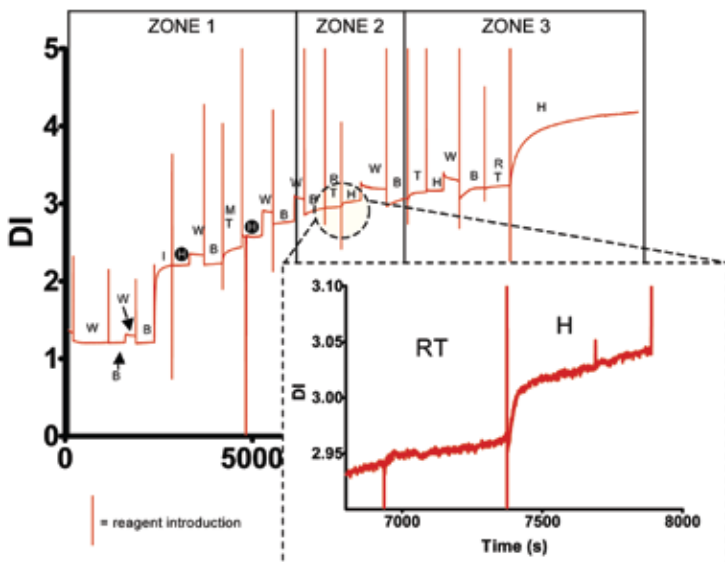
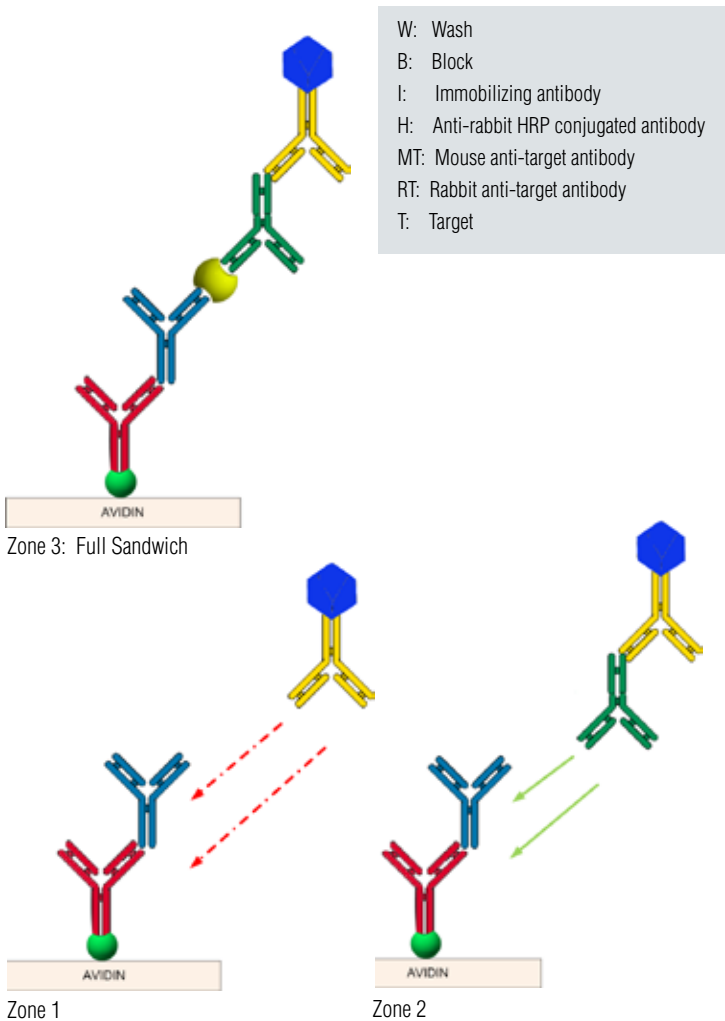


Figure 1: Sequential Addition of Multiple Immunoassay Components



Introduction

Multi-component assay systems are particularly prone to unwanted reagent interactions, which can generate substantial noise and background. An experiment showed substantial background in the absence of the target analyte indicating non-specific binding of HRP conjugated antibody (H). We devised a simple method of sequentially introducing assay components on the dotLab® System in the absence of analyte to determine this non-specific reagent cross reactivity.

Method

Multiple components of an immunoassay were introduced in a defined sequence in the absence of analyte to identify the source of cross reactivity causing background HRP activity (Figure 1).

Results

- The HRP conjugated antibody (H) did not display substantial binding to other components (black circles, Zone 1).
- The binding partner to H, rabbit anti-target antibody (RT), did bind spuriously to the immobilization antibody and/or the mouse anti-target antibody (Zone 2 and window).
- The marked increase in signal upon subsequent addition of the anti-rabbit HRP conjugated antibody (H) provides further evidence that the rabbit anti-target antibody is bound to the surface in the absence of target analyte.
- The contribution to background is relatively small as evidenced by the strong signal seen when target (T) is introduced followed by the addition of the rabbit anti-target antibody (RT) and the anti-rabbit HRP conjugated antibody (H) (Zone 3).

Conclusion

This simple method identified the source of spurious signal in a single experiment on the dotLab® System. Subsequent changes in assay parameters minimized its impact.