

Multiplex Immunoassays with Wide Dynamic Range Demonstrated

• Unprecedented dynamic range • Wide breadth of assay formats • Easy reagent optimization

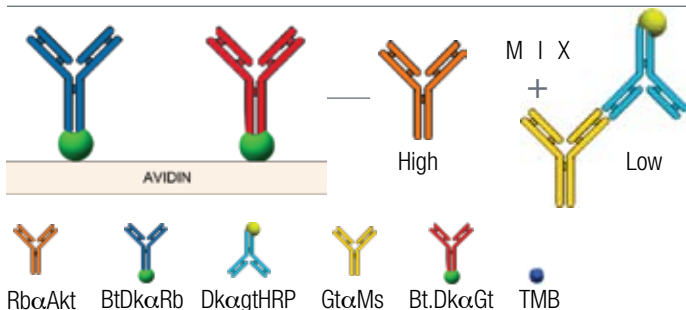


Figure 1. Multiplex Assay Format.

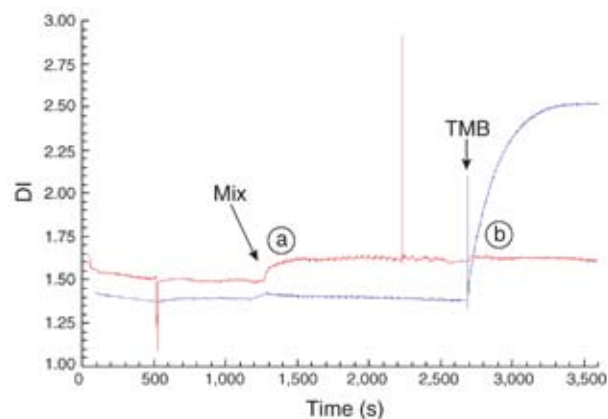


Figure 2: dotLab System Real-Time Trace of Multiplex Immunoassay.

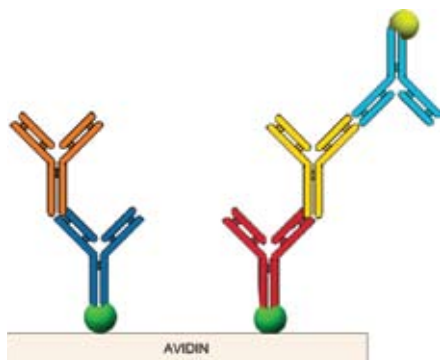


Figure 2a: red trace (a)

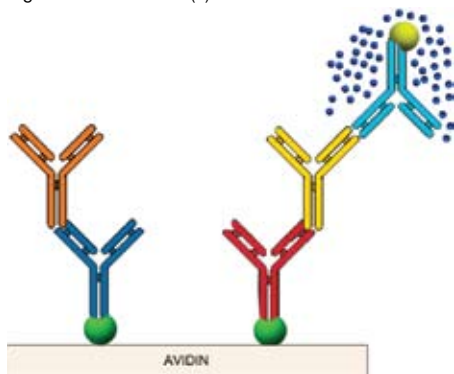


Figure 2b: blue trace (b)

Introduction

Multiplexing immunoassays has been inherently difficult due to cross-reactivity between reagents aimed at different analytes. Solutions are time-consuming, laborious, and expensive, with assays typically limited in dynamic range.

The dotLab® System overcomes these shortcomings by providing easy reagent optimization, multiple assay formats, and a broad dynamic range. Here we demonstrate the simultaneous quantitation of two similar analytes present at concentrations that differ by over six orders of magnitude.

Method

We used a mixture of:

- Rabbit anti-akt (RbαAkt) as the high-concentration analyte (10 µg/mL)
- Goat anti-mouse (GtαMs) as the low-concentration analyte (10 pg/mL)
- Horseradish peroxidase (HRP)-conjugated donkey anti-goat (DkαGt-HRP)

We immobilized two biotinylated (bt) donkey antibodies on two separate spots of an eight-spot streptavidin dotLab® Sensor; bt-DkαGt on one spot, and bt-DkαRb on another spot (Figure 1).

The antibody mix containing RbαAkt and GtαMs was loaded onto the sensor.

The binding events occurring at each of these locations were recorded and overlaid (Figure 2).

Results

A high-concentration of RbαAkt binding is detected by a rise in signal [Figure 2, red trace (a)].

GtαMs/DkαGt-HRP, in minute concentrations, provides no detectable signal and indicates no cross-talk.

The introduction of 3,3',5,5'-tetramethylbenzidine (TMB) precipitates in the presence of HRP conjugated to the DkαGt; thus, the low-concentration complex is detected. [Figure 2, blue trace (b)].

Conclusion

In this multiplex experiment, the dotLab System demonstrated the specific detection of the two analytes over six orders of concentration without signal cross-talk. This is made possible by the unique coupling of flow-based sensors, independent multiplex analysis, and real-time measurements. The dotLab System uniquely permits the development and use of new multiplexed panels, resulting in greater analytical utility.