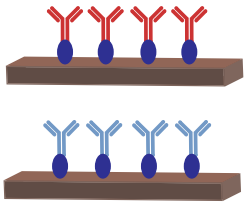
Extended Dynamic Range during Multiplex Analysis

One of the challenges facing multiplex immunoassays is the detection of analytes present over an extreme range of concentrations without the need for sample dilutions to cluster analytes into narrower concentration ranges. The broad dynamic range of the dotLab® mX System overcomes this problem to enable simpler multiplex assays on complex samples. The example below illustrates this feature in a duplex assay of two cardiac markers: NT-proBNP and C-reactive protein (CRP) at 25 pg/mL and 25µg/mL, respectively, spanning 6-orders of magnitude in concentration.



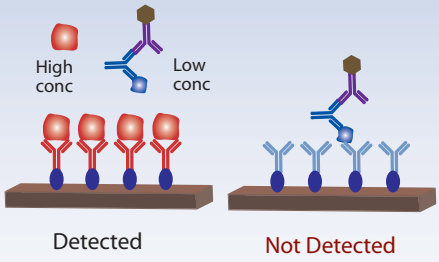
Biotinylated antibodies directed against CRP (red) and NT-proBNP (blue) were immobilized on two separate locations on an avidin sensor and then treated with the reagents listed below.

CRP

Goat anti-NT-proBNP

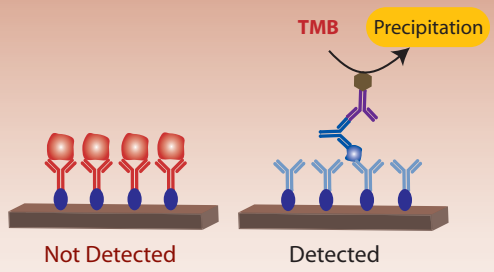
Donkey anti-goat-HRP

Step 1: Addition CRP and NT-proBNP-HRP complex

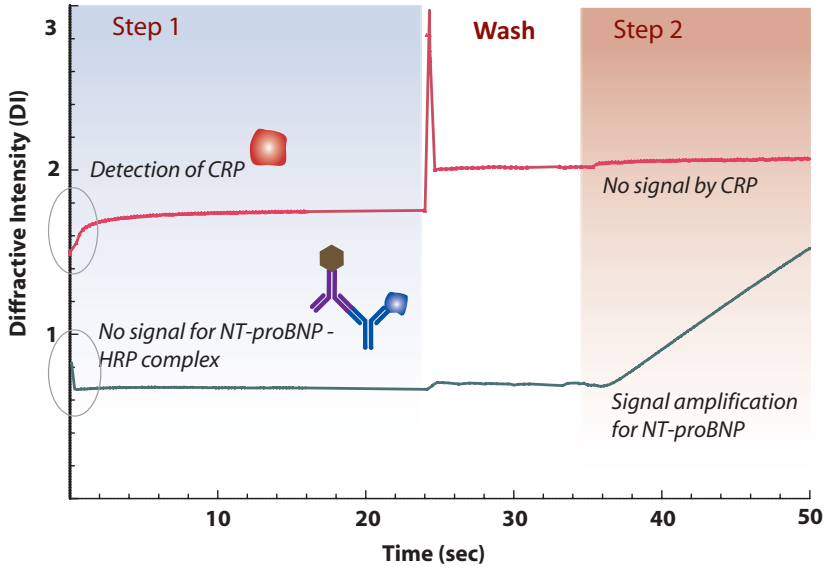


A mixture containing 25 µg/mL of CRP and 25 pg/mL of NT-proBNP as well as a Goat α-NT-proBNP and Donkey α-goat-HRP was loaded onto the sensor. The real time trace shows the signal for CRP binding but no signal for NT-proBNP due to its low concentration (see below).

Step 2: Addition of HRP substrate (TMB)



Addition of tetramethylbenzidine (TMB) results in a localized precipitation reaction with the HRP conjugated antibody captured on the NT-proBNP spot and a concomitant increase in signal. No signal change was observed at the CRP specific spot.



Highlights:

- A dynamic range covering 6-orders of magnitude was demonstrated using TMB signal amplification in the above duplex assay
- The alternative use of a tyramide signal amplification (TSA) technique in a separate NT-proBNP assay resulted in femtomolar detection levels at 0.1 pg/mL enabling a dynamic range of over 8-log units [see JF Houle, Combinatorial Chemistry & High Throughput Screening (2009), 12, 801-811]

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