

Development of a Multiplex Biomarker Assay

The development of multiplex assays is often complicated by cross reactions (or cross talk) between assay reagents and analytes. However, by using the panelPlus™ Sensor and real time analysis on the dotLab® mX System, potential cross talk issues can be clearly observed as demonstrated below with a set of ovarian cancer biomarkers (CA125, free bhCG and AFP). In this assay, antibodies for each target were conjugated to unique oligonucleotides corresponding to complementary oligonucleotides addressed at different spots on the panelPlus™ Sensor. When applied to the sensor, each of the antibody conjugates hybridized to their appropriate spots creating a multiplex capture surface (see Figure 1).

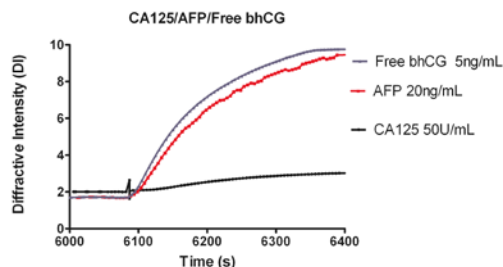
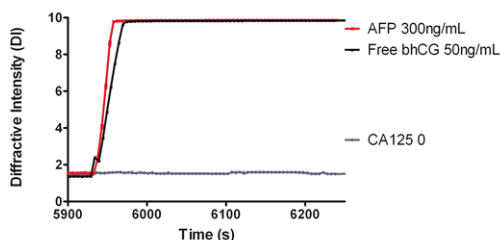
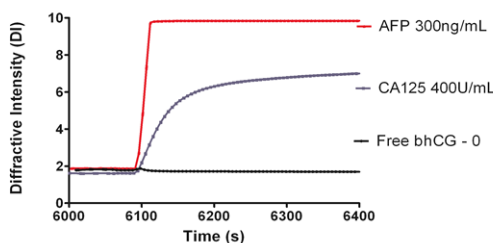
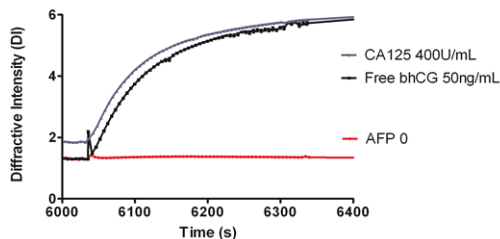


FIGURE 1

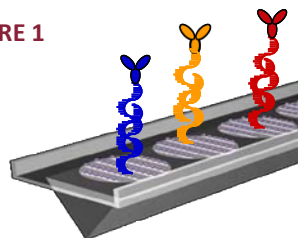


Illustration of oligonucleotide-conjugated antibodies for each target protein hybridized to their respective spots on the panelPlus Sensor.

- When high concentrations of CA125 and free bhCG were incubated on the panelPlus Sensor no detectable signal was observed on the sensor spot for AFP indicating that CA125 and free bhCG do not interact with the AFP antibodies bound to the sensor (top trace).
- Similarly, no cross reactivity was observed when high concentrations of CA125/AFP and bhCG/AFP were applied to the sensor bound bhCG and CA125 antibodies, respectively (second and third traces).
- A test serum was applied to the ovarian cancer panelPlus® Sensor showing the simultaneous detection of AFP, CA125 and free bhCG (bottom trace).

Highlights:

- The dotLab® System together with the panelPlus™ Sensors can be used to quickly develop multiplex biomarker assays
- Cross talk between assay constituents can be immediately identified by real time analysis