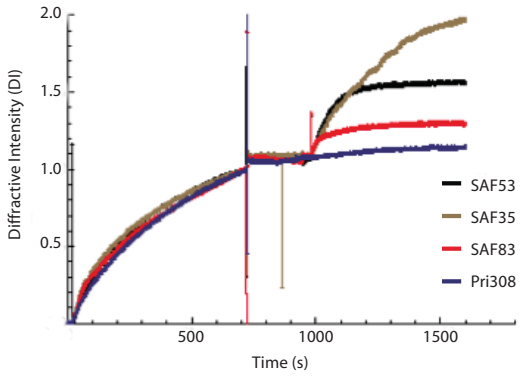
Rapid Development and Optimization of Immunoassays by Diffractive Optics

The development of an immunoassay often requires adjusting a large number of parameters including the choice of reagents and buffer conditions. Normally, these steps would require testing each parameter individually which can be very tedious and time consuming. However, the dotLab® mX System can aid in rapidly developing optimized immunoassay conditions by providing real time information on binding interactions and by its ability to sequentially probe an analyte or reagent. In the examples below, the dotLab® mX System was used to determine the proper detergent conditions and antibody pairs, and to identify possible cross reactive reagents.

Antibody Pairing

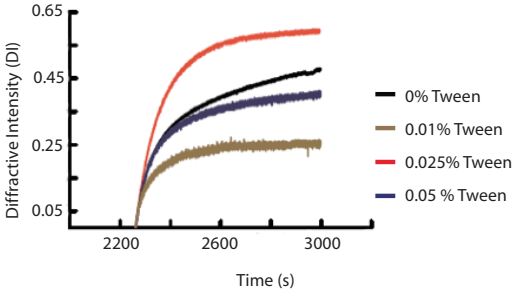


Four antibodies against recombinant human prion protein (PrPC) were evaluated for their ability to pair with biotinylated mouse monoclonal antibody 3F4.

PrPC was first pre-incubated with biotinylated 3F4 antibody (capture antibody) and then immobilized onto an avidin coated sensor.

Of the four antibodies, SAF53 was selected as the most optimal antibody due to its rapid time to equilibrium and its strong binding signal, whereas SAF32 would be ideal for a high sensitivity assay.

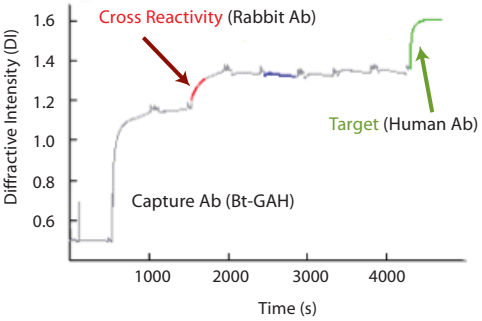
Detergent Optimization



In the same PrPC assay as above, the normalized results from four separate assays using various concentrations of detergent revealed that 0.01% Tween 20 gives the strongest binding signal and quickest time to equilibrium when SAF53 antibody was applied to the sensor.

Similar type of assays can be performed to determine optimal pH and salt concentrations.

Cross Reactivity



Significant cross reactivity was observed between rabbit antibody and the biotinylated goat anti-human capture antibody, whereas other reagents, including goat and mouse antibodies showed no cross reactivity to the capture antibody.

In less than 90 minutes the capture antibody was sequentially probed for cross reactivity against 6 different reagents and buffers.

dotLab® mX SYSTEM

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