

Assay Development: Antibody Pairing and Epitope Mapping

• Rapid antibody reagent selection • Affordable, fast, and easy • Informative real-time data • Epitope location information

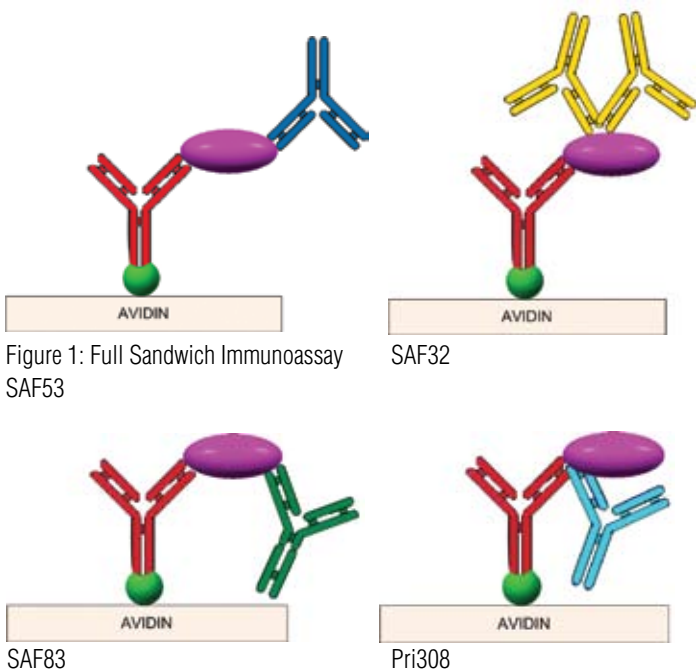


Figure 1: Full Sandwich Immunoassay
SAF53

SAF32

SAF83

Pri308

Introduction

Four antibodies against recombinant human prion protein (rhPrPc) were evaluated for their ability to pair with biotinylated mouse monoclonal antibody 3F4 (bt-3F4). The four antibodies — SAF32, SAF53, SAF83, and Pri308 — were analyzed in a full sandwich immunoassay using the dotLab® System (Figure 1).

The goal of this study was to select the best antibody for a fast and sensitive immunoassay for PrP detection. Antibodies were therefore ranked according to their ability to produce a high signal level quickly. In this effort, the real-time data generated epitope mapping information as well.

Method

A cocktail solution of 60 μ L of bt-3F4 (5 μ g/mL) and rhPrPc protein (5 μ g/mL) in BSA-DPBS was preincubated and immobilized onto a streptavidin dotLab® Sensor and then briefly washed on the dotLab System.

Each of the four candidate detector antibodies was introduced; binding was measured and real-time binding results were evaluated.

Results

The results of the four individual experiments were overlaid for comparison (Figure 2).

- SAF53 binds rapidly and equilibrates at a higher level than SAF83.
- SAF83 shows a rapid binding rate but lower signal at or near equilibrium. Subsequent experiments could include an analysis of off-rates.
- SAF32 shows a slower rate but higher signal without reaching equilibrium. Subsequent analysis has shown that this reflects its binding to the several octorepeat epitopes, and could be suitable for high sensitivity assays.
- Pri308 binds an epitope region that overlaps with 3F4. Virtually no binding was detected, so it would not be a suitable antibody pair with 3F4. However, this finding shows how the dotLab System can provide information on the relative epitope locations for the different antibodies.

Conclusion

SAF53 was selected as the antibody for the 3F4 full sandwich immunoassay because it provides the highest signal level quickly. The SAF53-3F4 assay therefore underwent subsequent optimization.

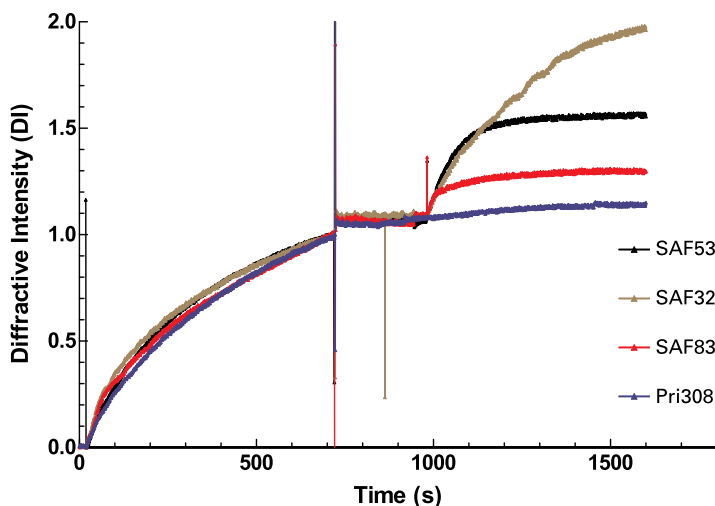


Figure 2: Detector Antibody Characterization