Simplifying Progress

Development of a Fab Arm Exchange ELISA for IgG4 Antibodies

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1. Introduction

Sartorius has developed a platform ELISA to measure Fab arm exchange (FAE) of IgG4 antibodies. IgG4 molecules are unique among the IgGs in that they can undergo FAE to form bispecific antibodies. Half-molecules of one IgG4 recombine with half-molecules of other IgG4 molecules to create antibodies with two specificities. This occurs in vivo and can also occur in vitro by adding mild reducing agents. There are two sites in the IgG4 molecules that are involved in determining the FAE: S228 in the core hinge and R409 in the CH3 domain. The ability of therapeutic IgG4 antibodies to potentially exchange with endogenous IgG4 antibodies in patients could lead to off- target effects. Some IgG4 antibodies, such as nivolumab, contain a mutation in the hinge region, S228P, that prevents this exchange from occurring. In contrast, natalizumab does not contain this mutation.

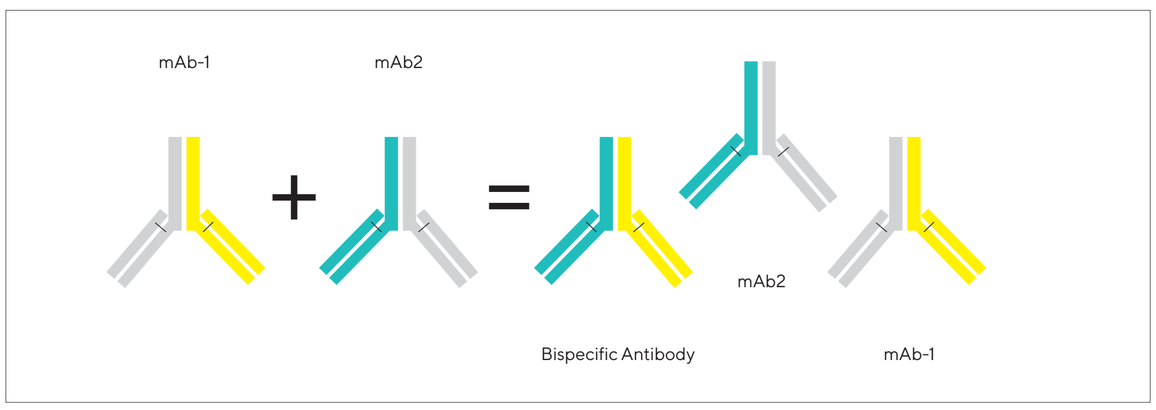

Figure 1: Schematic diagram of Fab arm exchange between two IgG4 monoclonal antibodies.

2. Experimental Setup

This assay utilizes a sandwich-based ELISA. By coating the plate with the target of one monoclonal antibody and detecting with an anti-idiotypic antibody against the second monoclonal antibody, the creation of any bispecific antibodies can be detected. Any monospecific antibodies remaining in the mixture will not be detected as they will either not bind to the target on the ELISA plate, or they will not be detected by the anti-idiotypic antibody.

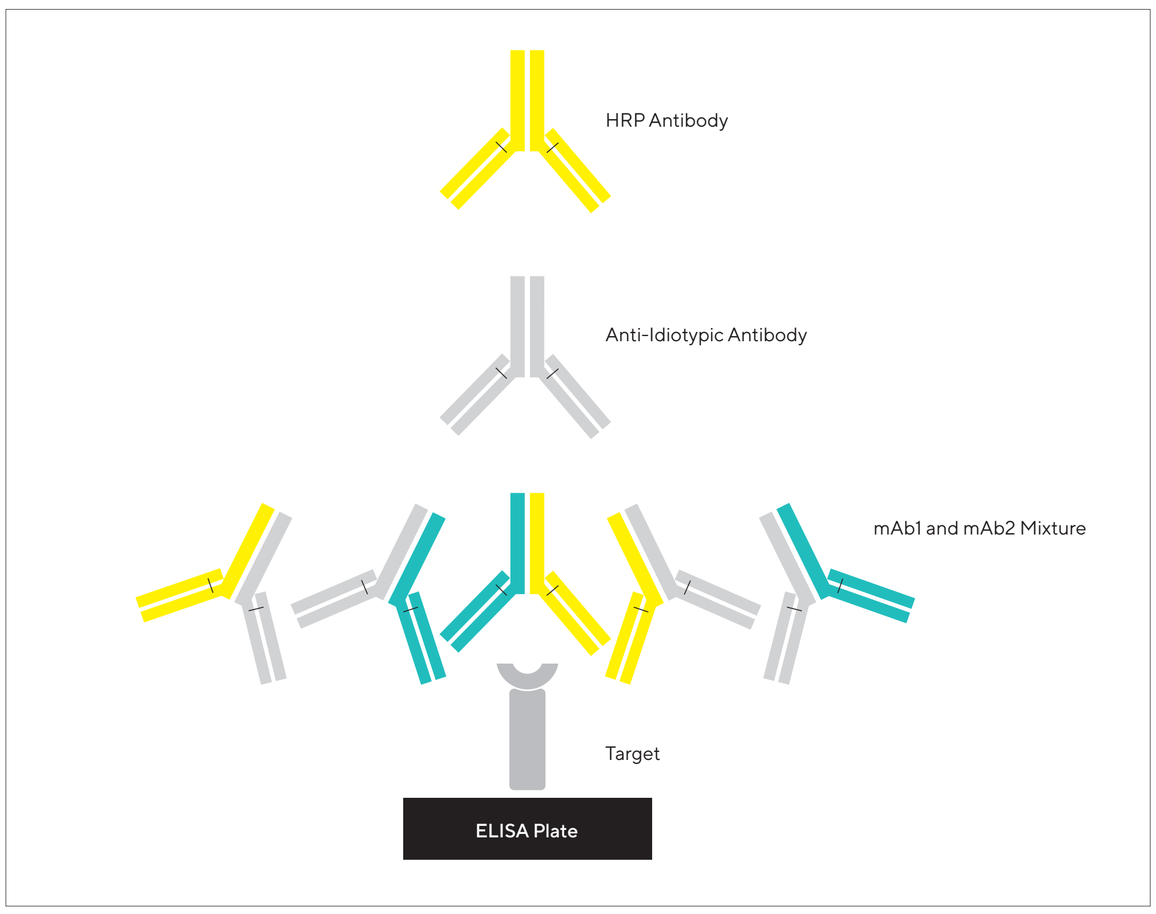

Figure 2: Schematic of the ELISA format to detect bispecific antibodies generated after Fab arm exchange.

3. Development using MODDE®

Using MODDE® Pro software, a full factorial design of experiment approach was used to begin development of the ELISA. There were two factors assessed: three concentrations of anti-idiotypic antibody and three concentrations of HRP antibody.

Experiment Name	Run Order	Anti-Idiotypic Antibody (concentration)	HRP (concentration)
N1	2	A	3
N2	3	B	3
N3	1	A	1
N4	4	B	1
N5	5	C	2
N6	6	C	2
N7	7	C	2

Table 1: Design of Experiments (DoE) full factorial run order with the seven runs anonymized. The concentration of the target remained constant throughout the different runs assessed.

4. Results – MODDE®

Output from MODDE® demonstrated the parameters required to achieve the optimum upper asymptote result. As shown in Figure 3, the lower concentrations of HRP result in the higher upper asymptote values. Figure 4 demonstrates the graphed data from Table 1, which was used to populate the information for MODDE® to run the analysis.

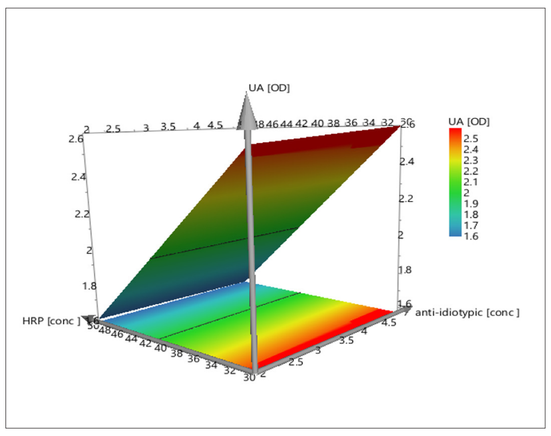

Figure 3: Response contour surface plot generated using the optimizer setting as determined by the software. Increasing anti-idiotypic concentration is shown on the X-axis, increasing HRP concentration on the Y-axis, and the upper asymptote results on the Z-axis.

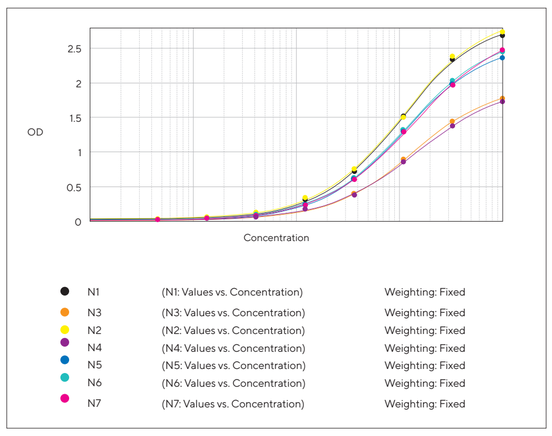

Figure 4: Graphed results of all seven DoE runs.

5. Results – Wild-Type IgG4 Antibodies

Two wild-type IgG4 antibodies without the S228P mutation were incubated with the reducing agent, Glutathione (GSH). They were subsequently tested in the ELISA shown in Figure 2. There was detection of bispecific antibodies in both the 5 mM and 0.5 mM GSH-treated samples, indicating Fab arm exchange had occurred.

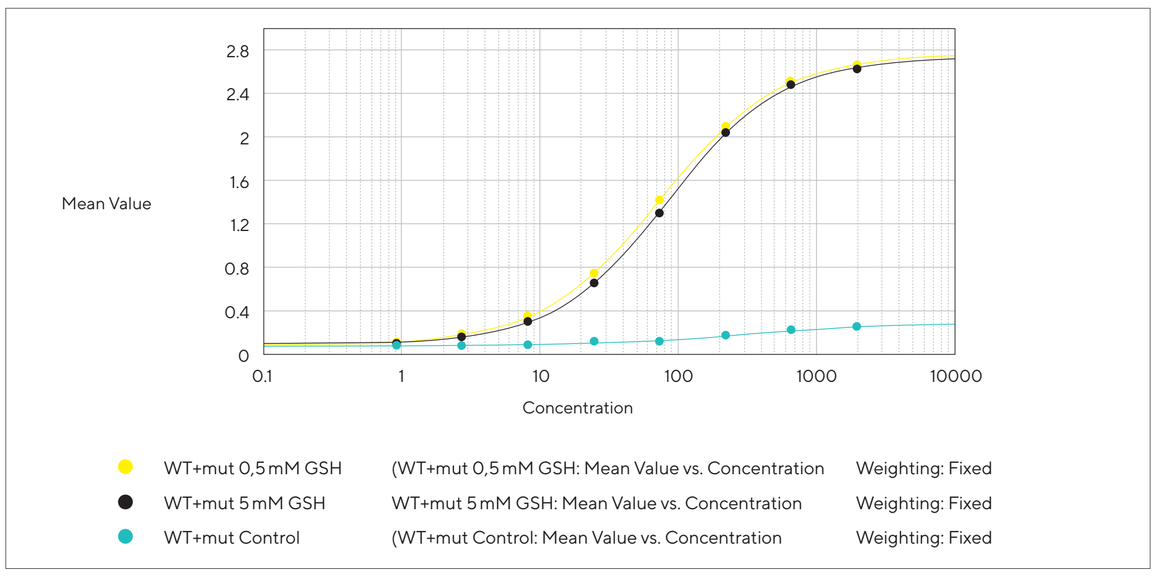

Figure 5: Graphed data of an ELISA testing two IgG4 antibodies without the S228P mutation.

6. Results – Mutated IgG4 Antibodies

One wild-type IgG4 antibody and one IgG4 with the S228P mutation were incubated with the reducing agent, GSH. They were subsequently tested in the ELISA shown in Figure 2. There is detection, it will occur for all IgG4 antibodies. Although the mutation is reported to stop Fab arm exchange, it is not uncommon to break some of the bonds at higher concentrations of reducing agent. The signal from these bispecific antibodies is also an order of 1.75 magnitude lower than the signal from the two wild-type antibodies. In addition, the signal from the 0.5 mM samples is at a similar level to the control sample, which has no reducing agent present.

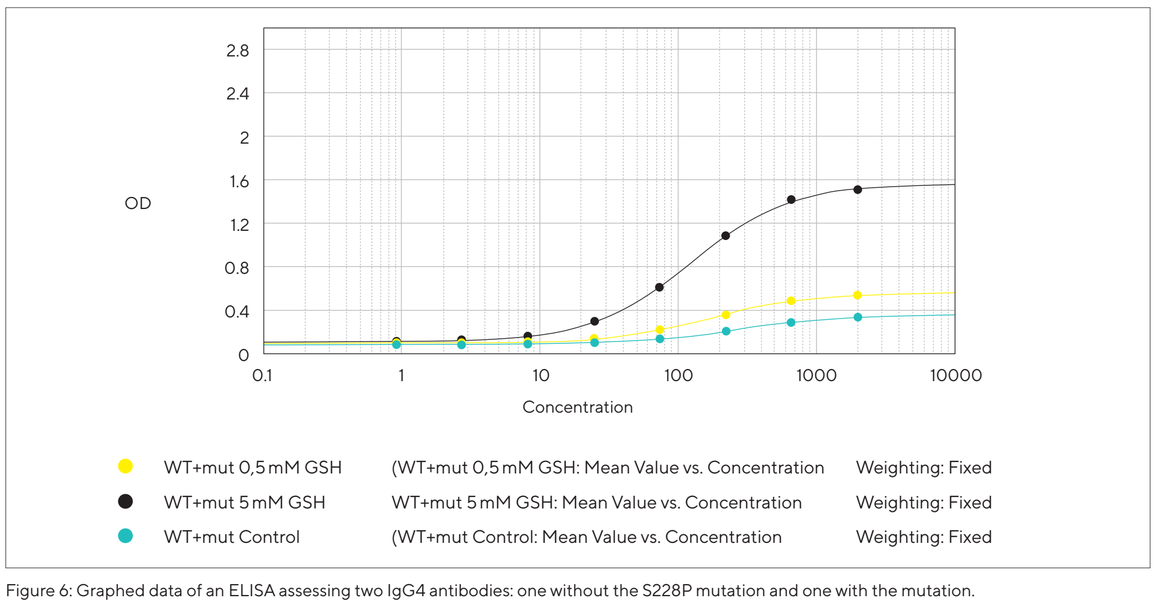

Figure 6: Graphed data of an ELISA assessing two IgG4 antibodies: one without the S228P mutation and one with the mutation.

7. Conclusion

The data presented here demonstrates that Fab arm exchange can be detected using an ELISA method. After incubation with reducing agents, the IgG4 antibodies create bispecific antibodies that can be exploited to detect both Fab regions. The plate is coated with one target, and detection of the other target is achieved by using an anti-idiotypic antibody, which results in the detection of these bispecific antibodies. The S228P mutation prevents this from happening to the same degree as the wild-type antibody, but it is still possible at higher concentrations of the reducing agent.