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Using the parallel configuration of Biacore™ 8K for efficient characterization of monoclonal antibodies

Åsa Sporrong, Linnéa Nygren Babol, Anna Moberg, and Anita Larsson

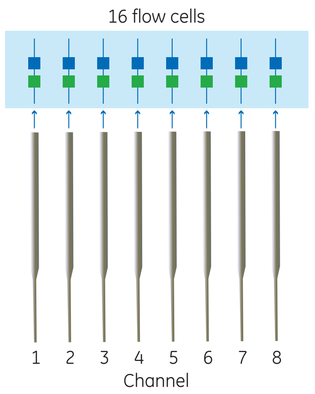
GE Healthcare Bio-Sciences AB, SE-75184 Uppsala, Sweden

Introduction

In this study, the possibilities of the parallel configuration of Biacore 8K (16 flow cells in 8 channels) for efficient reagent characterization was explored. As a model system, six different mouse monoclonal antibodies (mAbs) against recombinant GST (recGST) were tested and compared to a reference antibody.

The flexibility of 16 flow cells enables a customized assay set-up for each run. It is possible to group the runs as seperate activities in the *Activity queue* and execute them consecutively. The decision on selection of candidate mAbs and setup of the next activity in the *Activity queue* is taken after completion of the previous.

The reference antibody is a capture reagent for GST-tagged proteins and is used immobilized to a sensor chip.



Conclusions

- The parallel configuration of Biacore 8K enabled significant time savings in analyses of binding characteristics, activity, purity, and regeneration of mAb candidates.
- Flexible use of 16 flow cells allowed all analyses to be performed on a single chip.
- A mAb clone with binding characteristics against recGST and activity similar to the reference antibody was identified. However, the mAb was in an impure state.

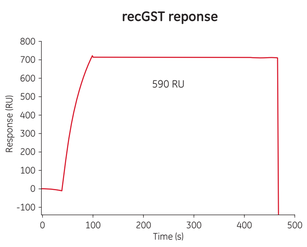
Experimental overview

The flexible, parallel approach included 11 runs grouped in 6 separate consecutive activities in the *Activity queue*, each having a decision point after completion.

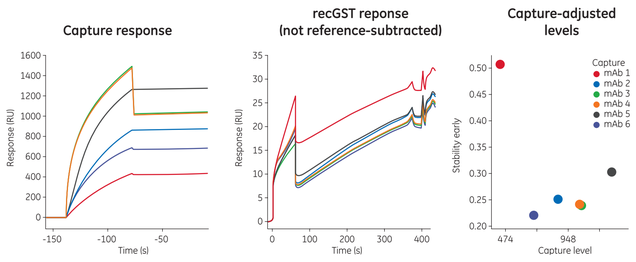
Activity no.	Run no.		Channel 1		Channel 2		Channel 3		Channel 4		Channel 5		Channel 6		Channel 7		Channel 8	
		Flow cell	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
1	1	Immobilization of Mouse Antibody Capture Kit (Ch 1, fc 1) and reference antibody (Ch 1, fc 2)	■	■														
	2	Binding test of reference antibody		■														
	3	Candidates mAb 1 to 6 tested for binding of recGST using Mouse Antibody Capture Kit	■															
		Select candidates with stable binding of recGST: mAb 1 and 6 selected																
2	4	pH-scouting of mAb 1 and 6			■		■											
		Select pH for immobilization of candidate 1 and 6: pH 5.0 selected for both mAb 1 and 6																
3	5	Immobilization of mAb 1 and 6			■	■	■	■										
	6	Test of maximal binding capacity of reference antibody and mAb 1 and 6		■		■		■										
		Select candidates with stable binding of recGST and promising binding capacity: mAb 1 selected																
4	7	Immobilization of anti-IgG_1, anti-IgG_{2a}, anti-IgG_{2b}, and anti-IgG_3 for test of mAb 1 preparation purity							■	■	■	■						
	8	Capture of mAb 1 followed by injection of recGST							■	■	■	■						
		Low purity revealed, decision to test immobilization at different conditions																
5	9	Immobilization of mAb 1 in acetate pH 4.0, pH 4.5, pH 5.0, and pH 5.5											■	■	■	■		
	10	Comparison of capacity to bind recGST											■	■	■	■		
		Select the most active surface																
6	11	Regeneration scouting of mAb 1														■		

1 *Test of binding properties*

Binding test of reference antibody (run 2)



Binding stability testing of mAb 1 to 6 on Mouse Antibody Capture Kit (run 3)

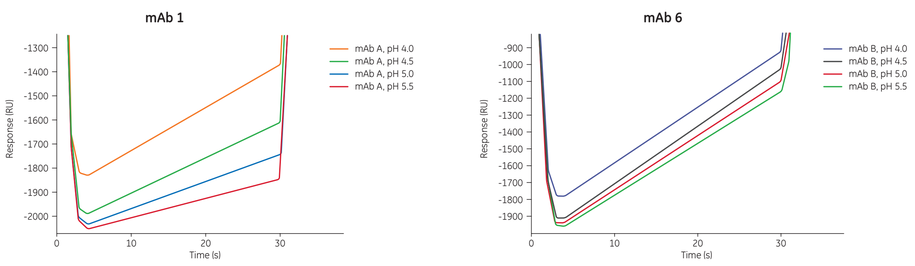


Stable binding, but low responses compared to the reference antibody was observed for all 6 mAbs.

➔ **mAb 1 and 6 were selected for further testing.**

2 *Test of conditions for immobilization*

pH-scouting of mAb 1 and mAb 6 (run 4)

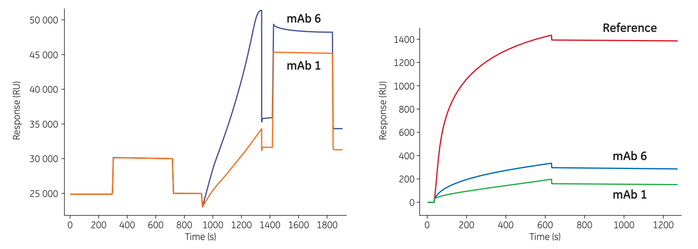


An atypical behavior was observed for both mAbs, indicating impure protein preparations. This was suspected to be due to contaminating polyclonal antibodies potentially originating from the manufacturing method.

➔ **pH 5.0 was chosen for immobilization of both mAb 1 and 6.**

3 *Test binding capacity on immobilized mAb*

Amine coupling of mAb 1 and 6 at pH 5.0 (run 5) and testing of the max binding capacity of mAb 1, mAb 6 and reference antibody (run 6)



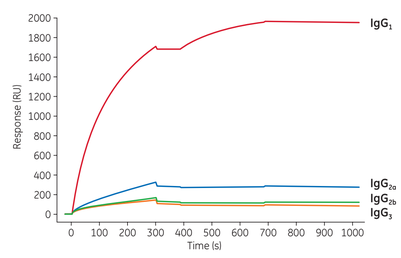
Ligand	Immobilized level (RU)	recGST response (RU)	Stoichiometric ratio (recGST/antibody)
reference	7700	1386	1.04
mAb 1	6400	299	0.27
mAb 6	9500	156	0.09

Stable binding, but low binding ratios were observed for both mAbs. Low activity/purity was suspected, confirming the results from run 4.

➔ **A decision to investigate purity was taken. mAb 1 was selected for further studies.**

4 *Test purity of mAb preparation*

Purity check using immobilized anti-subclass reagents. Injection of mAb 1 followed by recGST (run 8)



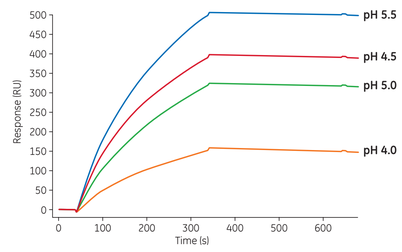
Capture reagent	Immobilization level (RU)	Ligand (RU)	Analyte response (RU)	Stoichiometric ratio (recGST/antibody)
Anti-IgG_1	9200	1684	275	0.94
Anti-IgG_{2a}	6900	283	7.5	–
Anti-IgG_{2b}	9100	128	-1.0	–
Anti-IgG_3	9700	106	-7.6	–

The presence of contaminating antibodies was confirmed. mAb 1 (of IgG_1 subtype) proved to be very promising demonstrating similar binding ratio as reference antibody (1.04).

➔ **A decision to test an approach to selectively immobilize mAb 1 from the impure preparation was taken.**

5 *Test binding capacity on immobilized mAb*

Immobilization of mAb 1 at different pH to test if mAb 1 can be selectively immobilized and enriched at a certain pH (run 9). Testing was made by injections of recGST (run 10)



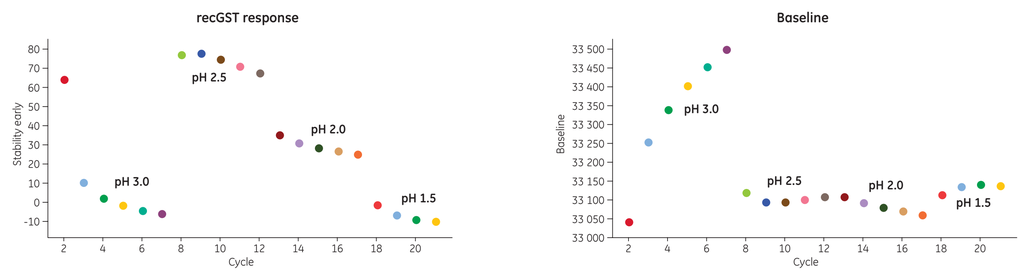
pH	Immobilized level (RU)	recGST response (RU)	Stochiometric ratio (recGST/antibody)
4.0	13 000	158	0.07
4.5	11 800	397	0.19
5.0	7100	324	0.26
5.5	8700	505	0.33

The selective immobilization approach was successful and the best binding capacity was obtained for mAb immobilized at pH 5.5.

➔ **Immobilization at pH 5.5 was selected for further testing of mAb 1.**

6 *Regeneration scouting*

Regeneration scouting with 10 mM glycine pH 3.0, 2.5, 2.0, and 1.5 (run 11)



None of the tested conditions worked well (note: starting level for recGST response [505 RU] is in run 10)

➔ **regeneration must be further optimized**

29215083 AA 06/2016