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Specific protein quantification during cell culture and process development using surface plasmon resonance

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Introduction

Quantification of protein is essential during pharmaceutical development, and a variety of methods and technologies for determination of total and specific protein concentration are available. To simplify practical aspects of analysis, we used the same platform for quantitation of different proteins of interest during process development as well as cell culturing (1). Here, we describe the development of a streamlined assay platform for specific quantification using surface plasmon resonance (SPR) technology.

Materials and methods

Biacore™ T200 systems were used for quantification of human IgG, IgG subclasses, albumin, IgA, and transferrin from plasma fractionation, and also for quantification of expressed monoclonal antibody from a Chinese hamster ovary (CHO) cell culture process. In Figure 1, the principle of a Biacore assay is exemplified for IgG quantification.

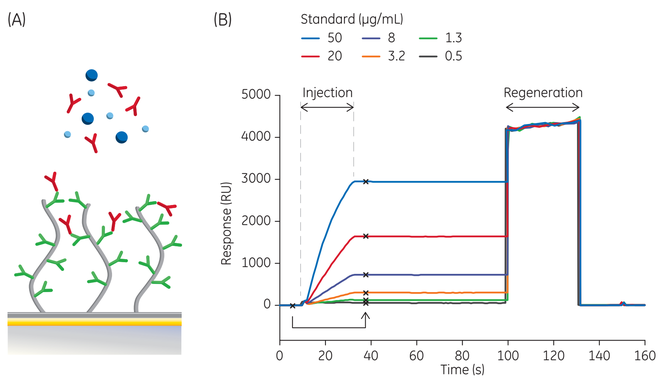

Fig 1. Biacore assay principle. (A) Anti-hIgG (green) is pre-immobilized on a Sensor Chip CM5 (a 30 min automated procedure using the Human Antibody Capture Kit). Thereafter, the sample is injected and IgG binds to the surface, resulting in a response. (B) Overlay plot showing injected IgG standard. Sample response levels are measured between report points (marked as ×) and plotted against concentration. After each injection, the surface is regenerated in preparation for a new injection.

Conclusions

- Specific protein quantification increased confidence in data.
- Robust assay: ~ 1000 process samples analyzed on the same surface.
- Master standard curves: in-process results in less than 10 min.
- Flexible assay formats using multiple flow channels.
- High sensitivity: impurities detected.
- Short hands-on time: 96- or 384-well format.

Reference

1. Frostell, Å., Mattsson, A., Eriksson, Å., Wallby, E., Kärnhall J., Illarionova, N.B., Estmer Nilsson, C. Nine surface plasmon resonance assays for specific protein quantitation during cell culture and process development. Analytical Biochemistry **477**, 1–9 (2015).

Results

Quantification of proteins from plasma process

The high robustness level of the assays enables the use of master standard curves (Fig 2). Thereafter, in-process results can be obtained in less than 10 min.

The Biacore assay for transferrin showed high level of robustness, and also high sensitivity with a limit of quantification (LOQ) of 27 ng/mL (Fig 3 and 4). Assay setup for IgG subclass distribution principle is shown in Figure 5. Compared with ELISA, similar results under shorter analysis time was achieved (Table 1). Table 2 summarizes Biacore quantification of in-process samples.

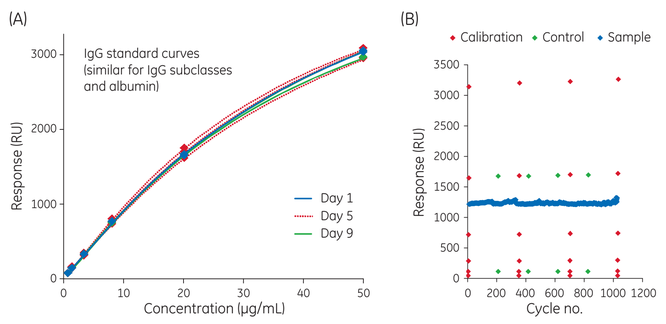

Fig 2. Assay performance over time. Analyses of more than 1000 IgG process samples were performed on the same surface over a time period of nine days. (A) Standard curves runs on day 1, day 5, and day 9 (a total of 6 curves). (B) Responses from an analysis with four standard curves, positive and negative controls, and repeated injections of the same sample resulting in a CV of 1.3% for the responses.

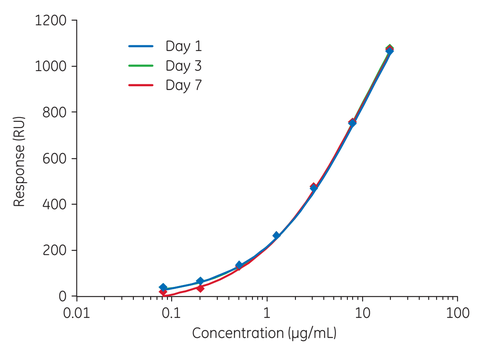

Fig 3. Transferrin standard curves from day 1, 3, and 7.

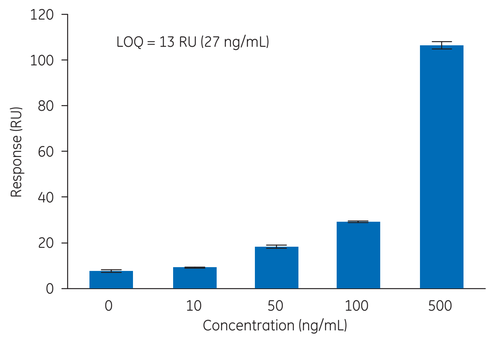

Fig 4. LOQ was determined as average response for [0 ng/mL] + 10 SD, n = 10.

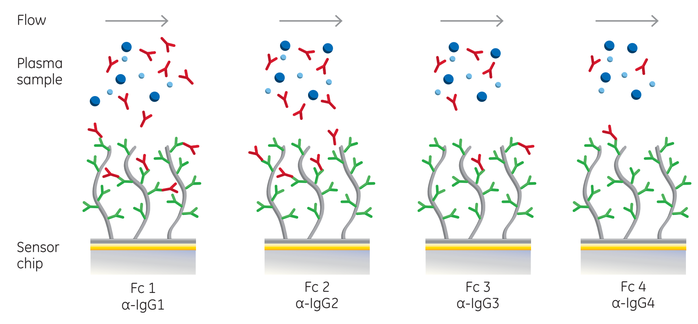

Fig 5. Schematic illustration of IgG subclass assay. Anti-hIgG antibodies against each subclass were pre-immobilized on the four surfaces of a sensor chip. Samples were run over all surfaces simultaneously. Each subclass interacts with its respective surface, generating four results in one experiment.

Table 1. Obtained concentrations and time for analyses

Control sample	IgG1 (%) (mg/mL)	IgG2 (%) (mg/mL)	IgG3 (%) (mg/mL)	IgG4 (%) (mg/mL)	Sum of IgG1–4 (mg/mL)	Hands on time		Total time	
						1 sample†	5 samples†	1 sample†	5 samples†
Biacore	63 10.7	36 6.1	1.2 0.2	0.18 0.03	17	40 min	1 h	2 h	6 h
ELISA*	54 7.4	44 5.9	0.9 0.1	1.2 0.2	14	2.5 h	4 h	5 h	6.5 h

*commercial ELISA for IgG subclasses
†2 replicates, 4 dilutions

Table 2. Summary of obtained calculated concentrations for eight different proteins from laboratory-scale testing of a plasma fractionation process

Purification step	IgG1 (%) (mg/mL)	IgG2 (%) (mg/mL)	IgG3 (%) (mg/mL)	IgG4 (%) (mg/mL)	Sum of IgG Sc (mg/mL)	IgG total (mg/mL)	Albumin (mg/mL)	Transferrin (µg/mL)	IgA (µg/mL)
Plasma	50 5.1	42 4.3	4.3 0.44	3.9 0.4	10.2	9.9	45	2534	1921
Sepharose™ 4 FF	51 2	41 1.6	4.3 0.17	3.9 0.15	3.9	3.8	18	1058	800
DEAE Sepharose FF	51 1.9	41 1.6	4.3 0.16	3.9 0.15	3.8	4	20	951	751
Euglobulin supernatant	58 2.9	34 1.7	4.4 0.22	4.1 0.21	5	5.2	25	1280	680
DEAE Sepharose FF	56 1.8	35 1.1	4.7 0.15	3.6 0.11	3.1	3.1	0.03	663	13
Q Sepharose FF	59 2	39 1.4	1.6 0.06	0.2 0.01	3.5	3.6	< 0.03	2.2	13
Sterile filtration	64 30	34 16	1.6 0.76	0.2 0.09	47	52	< 0.03	12	45

In agreement

Quantification of antibodies from cell culturing

Results from quantification of monoclonal antibodies during cell culturing are shown in Figures 6 and 7.

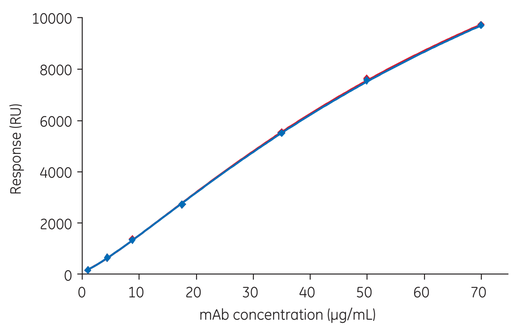

Fig 6. Standard curves of purified mAb binding to Sensor Chip Protein A. Overlay of two standard curves from fed-batch CHO cell culture. Up to 1000 antibody samples and standard injections can be run on the same chip. Samples injected at two dilutions typically show a CV < 2% in calculated concentration.

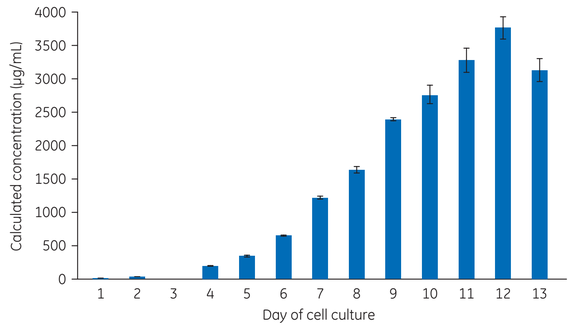

Fig 7. Concentration determination of a mAb using Human Antibody Capture Kit. Crude samples from each day of a CHO cell culture. Analysis of all samples (n = 270) was performed at 4 occasions during a time period of 12 days.

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