

Protein interaction analysis

Biacore™ Sensor Chip Protein A

Sensor Chip Protein A is a sensor surface pre-immobilized with a recombinant Protein A variant binding antibodies for protein interaction analysis using Biacore systems. The convenient, ready-to-use Sensor Chip Protein A has high binding capacity, offering a wide dynamic range with high reproducibility and robustness. Sensor Chip Protein A is an excellent surface choice for antibody concentration analysis in biopharmaceutical process development (e.g., cell line development and purification optimization), and in manufacturing QC. The sensor chip is also suitable for comparability studies and other types of characterization work involving kinetic analysis giving:

- **Savings in time and labor:** Ready-to-use sensor chip eliminates the need to develop immobilization and regeneration conditions
- **Confidence in data:** Highly controlled development processes and manufacturing conditions ensure reproducible results
- **Site-directed capture of antibody:** Binds only to the heavy chain within the Fc region and ensures antibodies are bound to the surface in a specific orientation

Description

The surface of Biacore Sensor Chip Protein A consists of a carboxymethylated (CM) dextran matrix with a recombinant Protein A variant covalently attached. The surface ligand is the same as is used for our MabSelect SuRe™ protein purification products meaning the same surface molecule can be used for purification and Biacore analysis in bioprocess development and manufacturing quality control. This recombinant Protein A binds antibodies from several mammalian species, most notably human antibodies of the subclasses IgG₁, IgG₂, and IgG₄ (Fig 2) but it does not bind to Fab fragments as native Protein A does.



Fig 1. Biacore Sensor Chip Protein A is available in Series S and Classic formats suitable for use with Biacore systems.

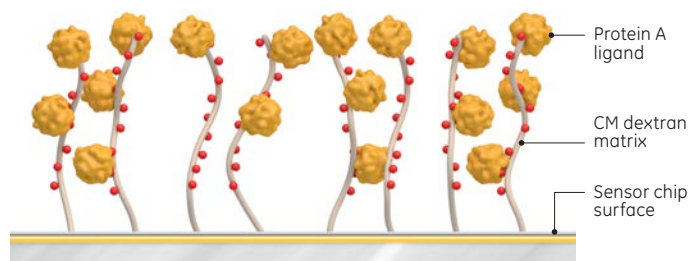


Fig 2. A recombinant Protein A variant is pre-immobilized to the sensor chip surface making it ready-to-use for concentration or interaction analysis with Biacore systems.

Applications

In concentration analysis, a sample or calibration solution is injected over the Protein A surface and the binding response is measured (Fig 3A). In affinity or kinetic analysis, an antibody is first captured on the Protein A surface followed by injection of analyte and any binding is detected (Fig 3B). Bound molecules are then removed from the Sensor Chip Protein A surface by regeneration with a glycine solution of pH 1.5, after which it is ready for a new analysis cycle.

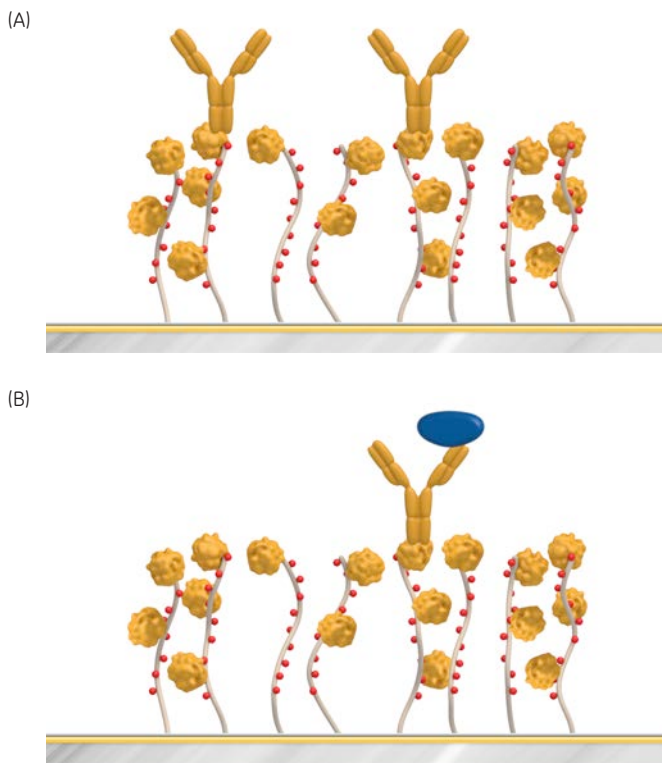


Fig 3. Surface of Sensor Chip Protein A used in (A) antibody concentration analysis, and (B) kinetic analysis of analytes (shown in blue).

Sensor Chip Protein A brings the convenience of having the surface pre-immobilized and ready to use. If higher surface flexibility (e.g., different molecules immobilized or different immobilization levels of the molecules in different flow cells) or other specificities are needed, use of our Human Antibody Capture Kit or Mouse Antibody Capture Kit together with Sensor Chip CM5, Sensor Chip CM4, or Sensor Chip CM3.

Concentration analysis LOD, LOQ, and dynamic range

A calibration curve of Avastin™, an IgG₁-based mAb for treatment of cancer that inhibits the growth factor VEGF-A, was run to determine the limit of detection (LOD), limit of quantitation (LOQ), and dynamic range. LOD and LOQ were calculated based on the buffer injection noise (+3 SD and +10 SD, respectively).

LOD was determined to be 1 ng/mL and LOQ was determined to be 8 ng/mL using 30 s injections. By increasing the injection time to 180 s, sensitivity was increased and both LOD and LOQ were lowered by a factor of four to 0.25 ng/mL and 2 ng/mL, respectively. A dynamic range from ng/mL to mg/mL was obtained (Figs 4 and 5).

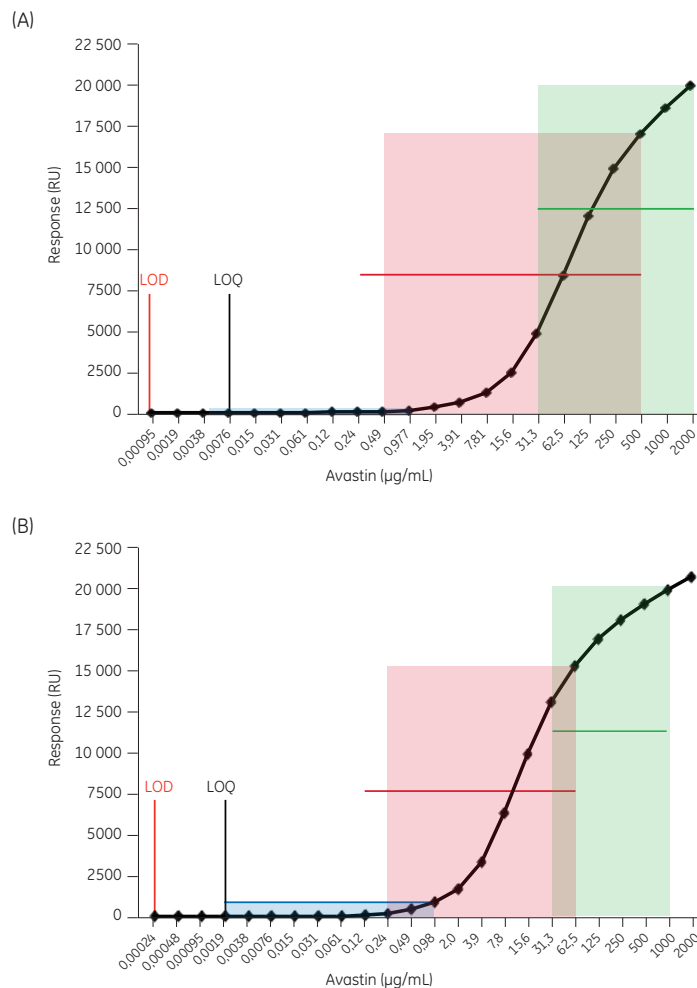


Fig 4. Measured responses for Avastin, (A) after 30 s, and (B) 180 s injection. The lines show concentrations used for creation of the calibration curve, where colored areas of the same color as the lines indicate concentration range with good fit to the constructed curve (< 10% deviation).

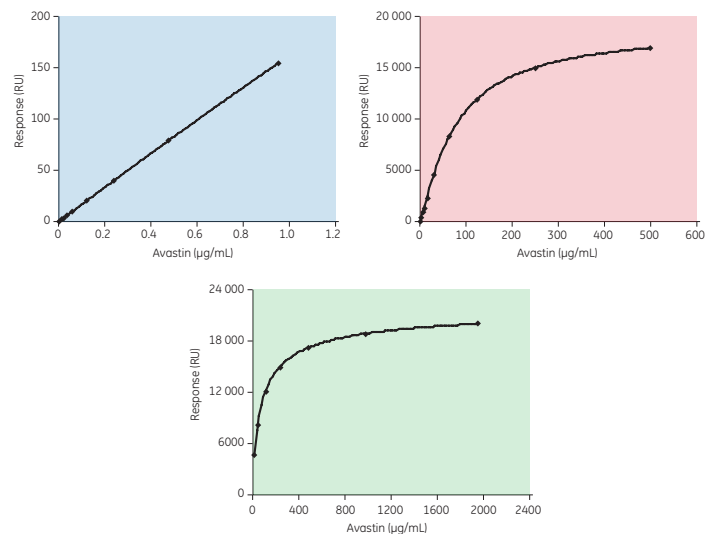


Fig 5. Calibration curves corresponding to the shaded concentration ranges in Figure 4A.

Precision

To demonstrate the use of Sensor Chip Protein A for IgG concentration analysis, a sample series from two fed-batch cultures of a mAb-producing CHO cell line was analyzed. Analyses were performed on two consecutive days in different flow cells on the same sensor chip, with sample preparation prior to each analysis. The samples were injected at two dilutions. A calibration curve (Fig 6) of purified IgG was used to determine the concentration of mAb from the CHO cell fed-batch cultures (Fig 7).

The experiment showed a coefficient of variation (CV) of $\leq 2.5\%$ within run and $\text{CV} \leq 4.0\%$ between runs for samples from the two cultures.

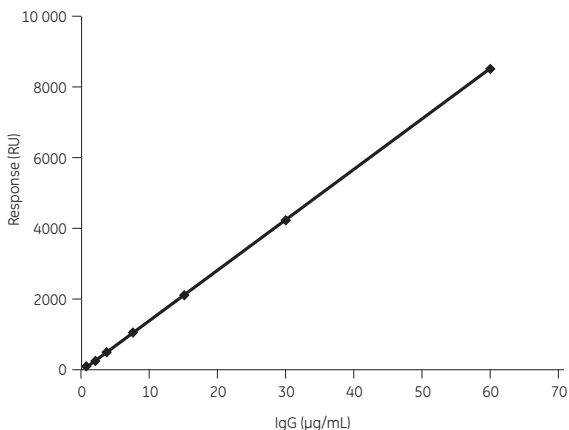


Fig 6. Calibration curve constructed from purified IgG in buffer.

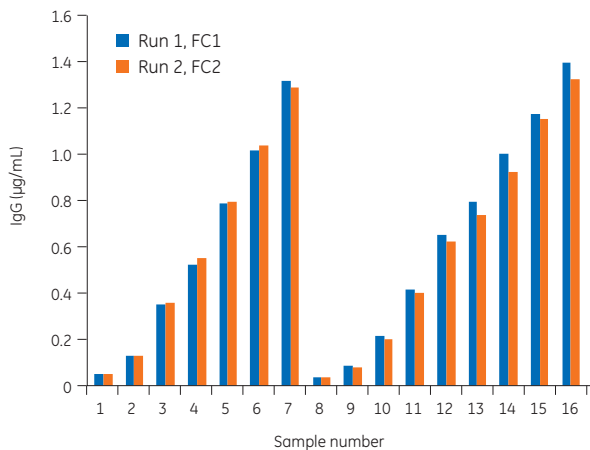


Fig 7. Measured IgG concentrations in a series of samples from two mAb-producing CHO cell fed-batch cultures (sample number 1 to 7: fed-batch 1, 8 to 16: fed-batch 2).

Table 1. Variation for sextuplicates of control samples in buffer at different concentration levels

Control sample (µg/mL)	CV (%) within run	CV (%) between runs
High (50)	0.1	0.7
Medium (25)	0.2	1.6
Low (2)	0.4	0.6

Kinetic analysis

Sensor Chip Protein A is optimized for concentration analysis, but is also useful in affinity and kinetic analysis involving antibodies. This is demonstrated in the following experiment where binding of TNF α (analyte) to the therapeutic antibody Infliximab (ligand) was measured.

Infliximab was captured to 1700 RU on Sensor Chip Protein A, followed by injection of TNF α at 3.125, 6.25, 12.5, 25.0, and 50.0 nM. Kinetic constants were determined to be: $k_{\text{on}} =$, $k_{\text{off}} =$, and $K_{\text{D}} =$ pM. The resulting sensorgram is shown in Figure 8.

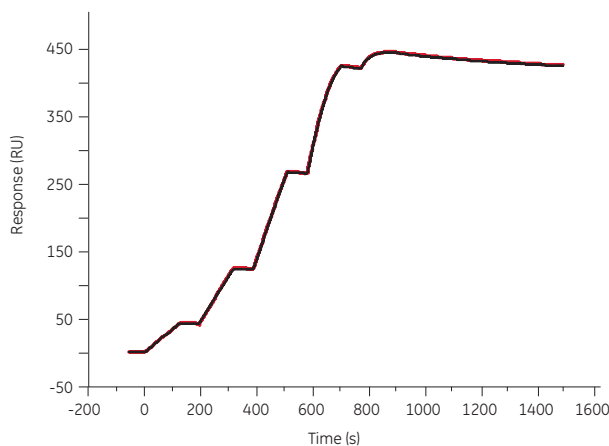


Fig 8. Sensorgram from analysis of Infliximab (ligand) and TNF α (analyte) in single-cycle kinetics mode on Sensor Chip Protein A. The sensorgram shows fitting of the data to 1:1 binding ($\text{Chi}^2 = 0.5 \text{ RU}^2$).

Ordering information

Product	Quantity	Product code
Series S Sensor Chip Protein A ¹	1	29127555
Series S Sensor Chip Protein A ¹	3	29127556
Sensor Chip Protein A ²	1	29127557
Sensor Chip Protein A ²	3	29127558

Related products

Glycine 1.5	1 × 50 mL	BR100354
Human Antibody Capture Kit	1	BR100839
Mouse Antibody Capture Kit	1	BR100838

Related literature

Sensor Surface Handbook	BR100571
Biacore Concentration Analysis Handbook	BR100512

¹ Used in Biacore 8K, Biacore S200, Biacore T200, Biacore T100, and Biacore 4000 systems.

² Used in Biacore X100, Biacore 3000, and Biacore C systems.

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