

Biacore™

Series S Sensor Chip Prisma

LABEL-FREE INTERACTION ANALYSIS

Analyze antibody concentration with Series S Sensor Chip Prisma and Biacore™ surface plasmon resonance (SPR) systems (Fig 1). The sensor surface is pre-functionalized with the same recombinant protein A molecule used to capture antibodies in MabSelect Prisma™ resins. Achieve high binding capacity and high reproducibility with this ready-to-use sensor chip. It offers a wide dynamic range to measure concentration and titer.

Use Series S Sensor Chip Prisma for antibody titer and concentration analysis in research, biopharmaceutical process development, and manufacturing quality control.

- Save time and effort with this ready-to-use chip. The pre-attached Prisma ligand means you don't have to develop immobilization and regeneration conditions for your antibody concentration assay.
- Have confidence in your data. Results are reproducible, because sensor chips are developed and manufactured under highly controlled conditions.
- Use one chip with a broad antibody binding specificity. The surface molecule binds between the constant heavy chain domains, C_H2 and C_H3, in the Fc region. It also binds the heavy chain within the Fab region.

Description

Series S Sensor Chip Prisma has a carboxymethylated dextran matrix that is pre-functionalized with a recombinant protein A (Fig 2). This protein is the same one used in Cytiva's MabSelect Prisma™ affinity chromatography resin, which is commonly used to develop and manufacture therapeutic antibodies. This recombinant variant on the surface of the sensor chip binds antibodies from several mammalian species, most notably human antibodies of the subclasses IgG₁, IgG₂, and IgG₄. Compared to Sensor Chip Protein A, the Prisma protein A ligand has enhanced binding affinity for the V_H3 sequence located on the variable heavy chain of the Fab region (Fig 3).



Fig 1. Biacore™ Series S Sensor Chip Prisma suitable for use with Biacore™ systems.

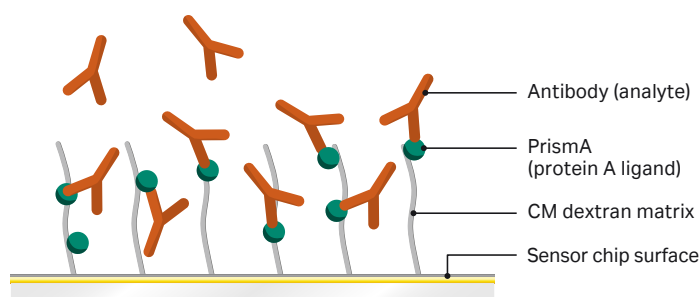


Fig 2. A protein A variant called Prisma is pre-functionalized to the sensor chip surface. As a result, the sensor chip is ready to use for concentration analysis with Biacore™ SPR systems.

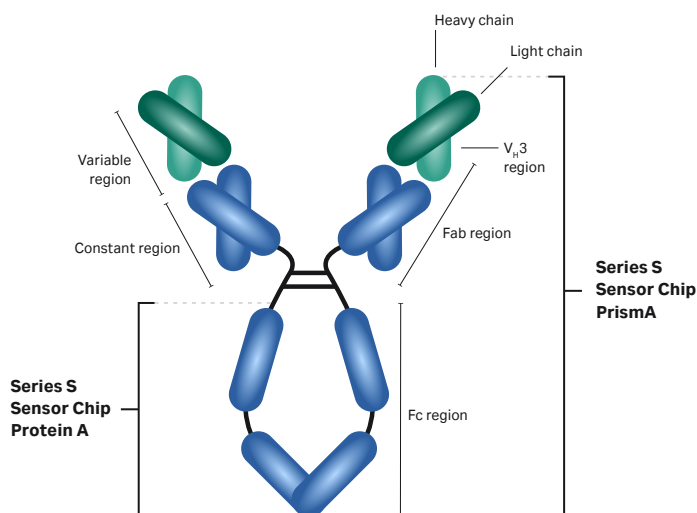


Fig 3. The binding of protein A mainly takes place between constant heavy chain domains, C_H2 and C_H3, in the Fc region of a monoclonal antibody (mAb). Compared to Series S Sensor Chip Protein A, Series S Sensor Chip PrismaA has enhanced binding affinity for the V_H3 sequence located on the variable heavy chain of the Fab region.

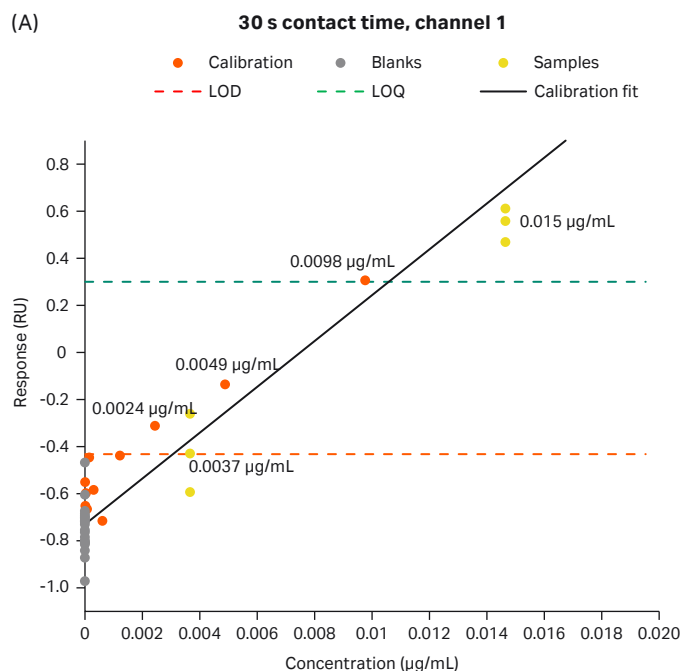
Applications

Series S Sensor Chip PrismaA is designed to bind human antibodies for titer or concentration analysis using Biacore™ systems. The ready-to-use sensor chip allows you to conveniently determine antibody concentration – using a calibration curve – in samples such as cell culture supernatants and chromatography fractions in cell line process development. It's also useful for quality control and product release testing. We don't recommend Series S Sensor Chip PrismaA for kinetic analysis due to the additional binding to the heavy chain within the Fab region.

Concentration analysis measuring LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) of an IgG₁ monoclonal antibody on Series S Sensor Chip PrismaA were determined to LOD = 3 ng/mL and LOQ = 10 ng/mL. LOD was lowered to 0.8 ng/mL and LOQ to 2 ng/mL by increasing the contact time from 30 s to 180 s. The exact limits will depend on both the specific antibody and the instrument used.

The LOD and LOQ are roughly in the same range as for Sensor Chip Protein A.



Dynamic range and concentration assay with antibody in buffer

The dynamic range of Series S Sensor Chip Prisma is $< 0.1 \mu\text{g/mL}$ to 1 mg/mL for a purified antibody sample in buffer solution. Calibration curves for an IgG₁ monoclonal antibody were constructed in three concentration intervals, chosen so that the deviation between the curve and the data is $\leq 10\%$. The same concentration intervals were used in a run using two channels on Biacore™ 8K+, except for the 180 s contact time run where the lowest concentration ($0.015 \mu\text{g/mL}$) was excluded due to poor fit of the curve (Fig 5).

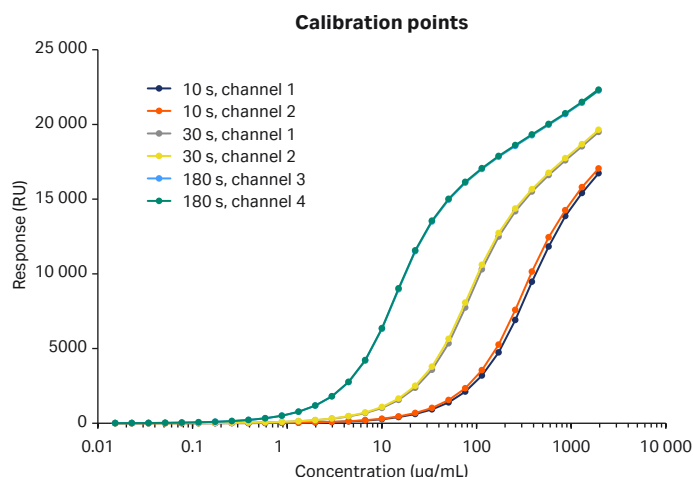


Fig 5. Calibration data points for 10, 30, and 180 s contact time, with connecting lines for guidance (not a data fit).

Concentration assay with samples from cell culture

Fed-batch cell culture supernatants containing secreted antibodies can be analyzed in a concentration assay described in the instructions for use.

You will likely see nonspecific binding (NSB) during upstream process development and concentration analysis of mAbs in cell cultures. This occurs when components present in the cell culture interact with the Prisma ligand; these effects can be reduced by dilution.

The exact dilution factor required will depend on the nature of your cell culture, but we recommend a minimum of 1:10. Dense cultures may require higher dilution factors to bring NSB down to negligible levels.

This is demonstrated by an experiment where the IgG titer in a mAb-producing fed-batch cell culture was calculated based on the binding responses of a supernatant sample diluted 1:10, 1:100, or 1:1000 in running buffer. As shown in Figure 6, 1:10 dilution was sufficient to get an estimate of the IgG titer in the culture. The concentration was determined more accurately by diluting the sample 1:100. For denser cultures, you may need up to 1:1000 dilution.

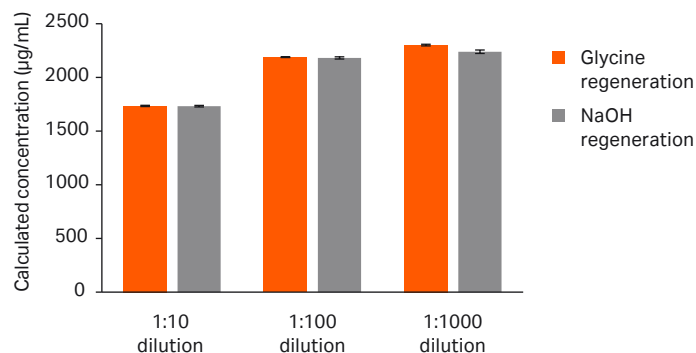


Fig 6. IgG concentration, average $\pm$ standard deviation of triplicates) in a mAb-producing fed-batch cell culture sampled on day 10. The concentration is calculated from the binding responses of samples diluted 1:10, 1:100, and 1:1000 in running buffer. Regeneration was done using a 30 s injection of 10 mM glycine pH 1.5 or a 120 s injection of 50 mM NaOH.

Downstream process optimization

You can use Series S Sensor Chip Prisma during downstream process development to determine the concentration of antibodies eluted from chromatography columns. Elutions from purification steps often have high ionic strength and/or low pH. To maximize binding to the ligand on the Series S Sensor Chip Prisma, you will need to dilute samples in running buffer before analyzing them.

Figure 7 shows the binding of the monoclonal antibody panitumumab (IgG₂) to Series S Sensor Chip Prisma under acidic conditions, chosen to represent typical chromatography elution buffers. Panitumumab was diluted to $10 \mu\text{g/mL}$ in HBS-EP+ running buffer, 50 mM acetate pH 4, or acetate buffer diluted 10-fold in running buffer. All samples were injected in triplicate, and the surface was regenerated with 10 mM glycine pH 1.5. The increased response seen at low pH is likely an effect of pre-concentration at the dextran surface. Note that the binding level is restored to within 10% of the control value when the acetate buffer is diluted 10-fold.

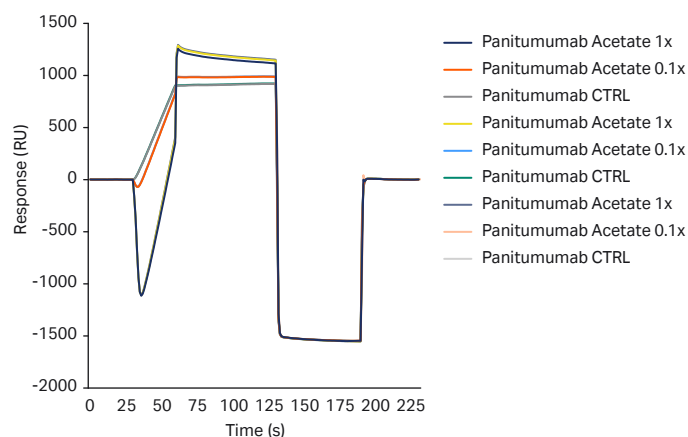


Fig 7. Sensorgram showing the binding response of a 30 s injection of panitumumab in running buffer ("CTRL"), 50 mM acetate pH 4 ("Acetate 1x") or acetate buffer diluted 10-fold in running buffer ("Acetate 0.1x").

Cost-efficient surface for concentration analysis

To demonstrate the surface stability of Series S Sensor Chip Prisma, a concentration assay with 200 cycles in one flow cell was performed. Figure 8 shows the assay calibration curves, included every 100th cycle in this example.

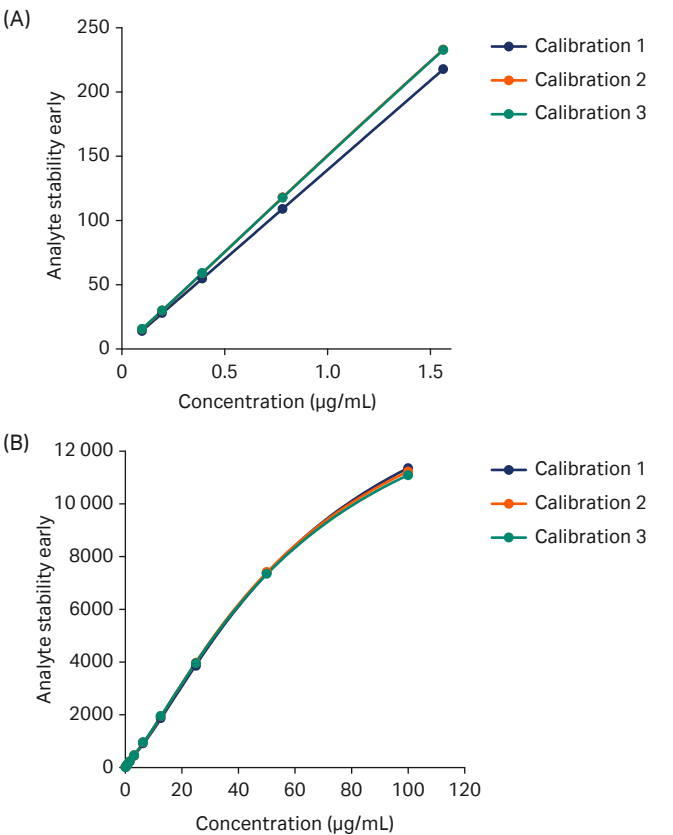


Fig 8. Calibration curves run at start (Calibration 1), middle (Calibration 2), and end (Calibration 3) of the assay (i.e., every 100 cycles), split into (A) a lower (0.1 to 1.6 g/mL) and (B) a higher (3.1 to 100 µg/mL) range.

Antibody control samples at three different concentrations were injected in quintuplicate at the start and at the end of a 200-cycle run (i.e., a total of 10 injections per control sample). The precision was < 5% coefficient of variation (CV) on read concentrations (Table 1).

Table 1. Coefficient of variation on read concentration for control samples at different concentration levels. Samples were injected as quintuplicates at the start and end of a 200-cycle run. The average of three calibration curves (run at the start, middle, and end of the assay) was used

Control samples	%CV within run
High (37.5 µg/mL)	0.6
Medium (4.7 µg/mL)	2.2
Low (0.3 µg/mL)	3.7

Ordering information

Product	Quantity	Product code
Series S Sensor Chip Prisma	1	29650263
Series S Sensor Chip Prisma	3	29650264

Use in Biacore™ 8K, Biacore™ 8K+, Biacore™ S200, and Biacore™ T200 SPR systems.

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