Efficient assays with orientation-specific binding of antibodies

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Introduction

Many researchers are facing increasing challenges in assay development working with complex sample matrices, difficult targets, and often with the need to produce results at a faster pace. Antibody applications are supported by a number of Biacore™ sensor chips and reagents enabling rapid assay development. This poster presents three newly developed pre-immobilized sensor chips: Sensor Chip Protein A, Sensor Chip Protein G, and Sensor Chip Protein L. These sensor chips enable orientation-specific binding of antibodies for subsequent antibody concentration and other analyses and have a high binding capacity. The sensor chips use the same recombinant surface ligands as for GE’s chromatography-based purification products.

Future Biacore consumables development follows a product category called Biacore Extend, with the purpose of development of sensor chips and reagents at a faster pace than previously for researchers working in nonregulated environments.

Biacore Sensor Chip Protein A

The surface of Sensor Chip Protein A consists of a carboxymethylated dextran matrix with a recombinant Protein A variant covalently attached. Sensor Chip Protein A has the same surface ligand as is used for MabSelect SuRe™ protein purification products. This recombinant Protein A variant binds only to the heavy chain within the Fc region, which ensures a specific orientation of the antibody on the surface. It may also be useful in antibody purification optimization since it does not bind to Fab fragments, as native Protein A does. Antibodies from several mammalian species bind to the surface, most notably human antibodies of the subclasses IgG1, IgG2, and IgG3. Sensor Chip Protein A is suitable for concentration analysis and characterization work involving kinetic analysis.

Concentration analysis

High sensitivity and wide dynamic range

Limit of detection (LOD), limit of quantitation (LOQ), and dynamic range were determined using a calibration curve for Avastin™ IgG1, based mAb for the treatment of cancer, which exhibits the growth factor VEGFA. A dynamic range from 90 ng/mL to 90 μg/mL was obtained (Fig. 1). The colored lines show the precision and sensitivity of the concentration curves. Colored areas of the same color as the lines indicate good fit to the calibration curves, 95% confidence interval (LOD and LOQ) were determined at 30 and 180 s injection time, both were approved LOD injection time (Fig. 1).

Kinetic characterization example

Tumor necrosis factor alpha (TNF-α) binding kinetics to mAb5 was analyzed. Samples of mAbs from CHO cell perfusion culture were compared with mAbs from fed-batch culture, and pushed mAbs, and infliximab (a chimeric monoclonal antibody drug). Perfusion culture samples were collected from 13 d of cell culturing, after cell density had reached steady state. Similar binding kinetics were observed over the 13 d of perfusion culture. Purification did not significantly alter binding kinetics (Fig 3). All tested antibodies had very high affinity (Kd < 10 nM) and were stable over the highest efficacy (Fig 3), and binding was very stable with low dissociation rate (R2 and Fig 5). Data was on excellent fit to a 1:1 binding model.

Biacore Sensor Chip Protein G

~ a Biacore Extend product

The carboxymethylated dextran matrix on Sensor Chip Protein G is pre-immobilized with a recombinant Protein G–Gammabind™, Type 2 — which is the same molecule that is used in some protein purification lab reuses from GE. The recombinant Protein G binds to a broad range of IgGs, such as human (including IgG1, IgG2, IgG3, IgG4, IgM, IgA, IgG2a, IgG2b, and IgG2c). Antibodies bind to the heavy chain within the Fc region ensuring antibodies are bound to the surface in a specific orientation. This is a sensor chip for antibody quantitation and characterization in a wide range of applications.

Biacore Sensor Chip Protein L

~ a Biacore Extend product

The carboxymethylated dextran matrix on Sensor Chip Protein L is pre-immobilized with a recombinant Protein L. Sensor Chip Protein L has the same surface ligand as used for Capto™ L protein purification products from GE. The recombinant Protein L binds to the human variable domain of kappa light chain for Capto™ L protein purification products from GE. The recombinant Protein L binds to the human variable domain of kappa light chain from other species such as mouse, rat, and pig but not to bovine IgG. Sensor Chip Protein L can be used for capture of antibody fragments that lack the Fc region: ScFv, Fab, and Fab′.

This is a sensor chip for antibody and antibody fragment quantitation and yes/no screening.

Biacore Extend products

Biacore Extend products are early access tools providing researchers working in nonregulated environments with the convenience of a wider choice of consumables optimized for Biacore applications. Utilizing the same high-quality components used for the manufacture of standard Biacore consumables, they enable faster and simpler development of a broader range of assays while retaining the high performance levels your work demands.

What to expect from Biacore Extend products:

• Ordered and supported as standard products
• Instructions for use are found at genesciences.com/instructions
• QC performed, but no Certificate of Analysis/Conformance issued
• Reduced product information and application examples
• Lot-to-lot variation might be higher compared to standard products
• For some products the delivery time might be up to 8 wk