PROTEIN INTERACTION ANALYSIS

ProteOn™ Sensor Chips

- Specific to the ProteOn XPR36 system
- Ideal for kinetic studies of multiple unlabeled biomolecular interaction partners
- Efficient ligand immobilization to ensure optimal activity
- Enclosed in bar-coded cartridges for reliable data tracking and archiving

Novel Surface Chemistries — Improve Your Surface Plasmon Resonance (SPR) Workflows

A Variety of Surface Chemistries for Specific Applications

Used with the ProteOn XPR36 protein interaction array system, ProteOn sensor chips are built with an alginate polymer matrix bound to a thin gold film on a sensor prism. The alginate matrix can be functionalized with several different reactive groups to achieve a variety of immobilization surface chemistries. The hydrophilic nature of the alginate layer creates a solution-like environment that prevents denaturation of the immobilized ligand and nonspecific adsorption of the analyte. The surface chemistry of the ProteOn sensor chips allows the ProteOn system to detect tight binding interactions down to picomolar concentrations of analytes, or analytes as small as 95 dalton. The combination of ProteOn sensor chips with the unique 6 x 6 interaction array allows the interaction analysis of up to 36 separate ligand/analyte pairs on a single chip, thereby increasing the throughput of a single experiment.

ProteOn Sensor Chip Performance

ProteOn sensor chips* provide reliable real-time analysis of protein-protein, protein-peptide, protein–small molecule, protein–nucleic acid interactions, and lipid-based applications with the following advantages.

- High ligand activity and stability
- Outstanding kinetic and equilibrium analysis
- Sufficient sensitivity for detection of low-molecular weight analytes (>95 dalton)
- Spot-to-spot reproducibility
- Storage stability

* ProteOn sensor chips are packaged with an inert gas in a sealed pouch and should be stored at 4°C. It is important to allow the sensor chips to reach room temperature before opening the pouch. The ProteOn sensor chips are continually monitored for quality to guarantee spot-to-spot reproducibility within the 6 x 6 array.

BIO-RAD

Novel Surface Chemistries

There are seven types of ProteOn sensor chips that can be used for a variety of different immobilization strategies and for the creation of different capacity surfaces.

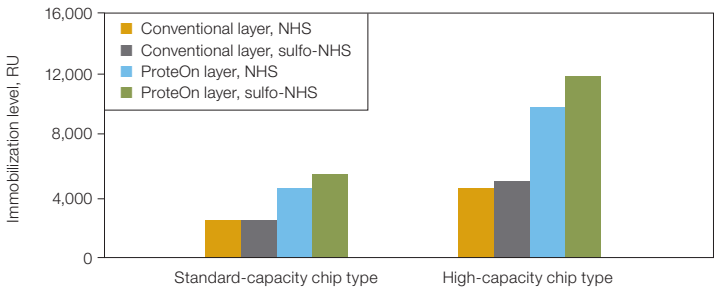
1. The GLC, GLM, and GLH sensor chips are amine coupling sensor chips with different ligand immobilization capacities. Ligands may be amine coupled to these sensor chips at different densities by controlling the amount of ligand injected, tuning the surface activation level, or adjusting the length of time of the immobilization step.
2. The NLC and HTG or HTE sensor chips are designed for site-specific capturing of biotinylated targets or histidine-tagged proteins, respectively.
3. The LCP sensor chip, used with the LCP capturing reagent kits, is designed for capturing lipid assemblies such as liposomes and lipoparticles.

The ProteOn sensor chips provide novel surface chemistries to improve existing SPR workflows.

- Amine coupling surface chemistry (GLX sensor chips) — featuring easily activated carboxyl groups for simple activation protocols and high activation rate
- NeutrAvidin surface chemistry (NLC sensor chip) — featuring NeutrAvidin for efficient capturing of biotinylated ligands
- Tris-NTA surface chemistry (HTG or HTE sensor chips) — featuring tris-NTA functional groups for stable and regenerable capturing of histidine-tagged proteins
- Planar NeutrAvidin surface chemistry (LCP sensor chip) — featuring the LCP capturing reagent kit for easy and regenerable capturing of lipid assemblies

Innovative ProteOn Surface Chemistry for Optimized Ligand Activity

ProteOn sensor chips are prepared with a modified alginate polymer layer bound to the gold surface of the sensor prism. This layer ensures optimized ligand immobilization for high signal. ProteOn sensor chip surface chemistry also optimizes the amount, net negative charge, and stereochemistry (therefore active binding sites) of ligand proteins. The result is user-controlled sensitivity down to the sub-nanogram level with heightened signal and greater response (RU) for protein-analyte experiments. Several varieties of application-specific sensor chip surface chemistries further optimize experimental results. After analysis, the sensor chip surface is easily deactivated to minimize any residual surface charge and nonspecific interaction with subsequent analytes.



Comparative coupling efficiency. Representative data for immobilization of rabbit IgG. Ligand coupling efficiency of ProteOn's easily activated layers is higher than conventional layers, and activation of ProteOn layers is higher using sulfo-NHS instead of NHS.

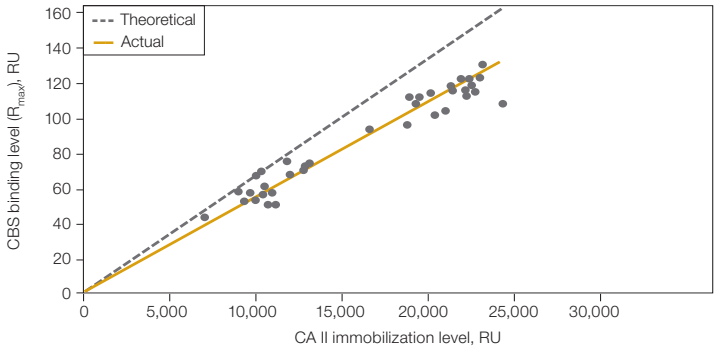
Representative immobilization efficiencies on ProteOn sensor chip surfaces designed for high protein binding capacity.

Protein	pI	Non-Bio-Rad Chip, NHS Activation, RU	GLM Chip, NHS Activation, RU	GLM Chip, Sulfo-NHS Activation, RU	GLH Chip, Sulfo-NHS Activation, RU
Pepsin	3	70	750	2,050	2,470
Ovalbumin	4.5	2,800	3,400	6,700	6,800
Protein A	5.1	4,300	3,500	6,000	18,800
β2-microglobulin	5.3	2,600	3,250	3,650	12,400
Carbonic anhydrase II	5.9	6,600 ±2,300	6,000	9,000	21,200
Myoglobin	6.9–7.4	3,900	2,800	7,000	12,200
Polyclonal IgG	6–8	10,000	9,700	12,200	22,200

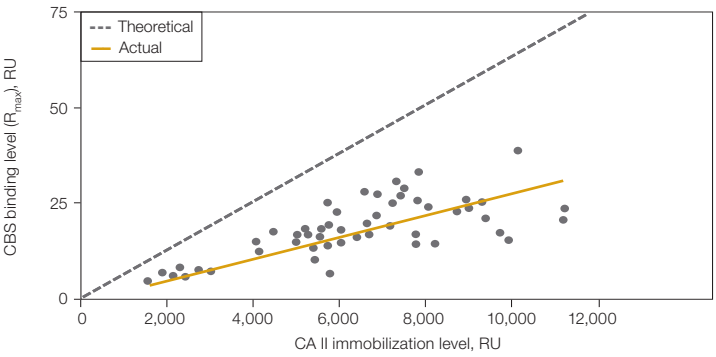
Comparative binding data for TEM1 KSSR/BLIP and carbonic anhydrase II/CBS interactions. The use of sulfo-NHS instead of NHS contributes to higher ligand binding, ligand activity, and analyte signals.

	k_a, $M^{-1}sec^{-1}$	k_d, sec^{-1}	K_D, M	Ligand Binding, RU	R_{max}	Ligand Activity, %
TEM1 KSSR/BLIP						
NHS	3.37×10^3	3.49×10^{-3}	1.04×10^{-6}	600	21	20
Sulfo-NHS	3.72×10^3	3.00×10^{-3}	8.06×10^{-7}	1,098	103	55
Carbonic Anhydrase II/CBS						
NHS	2.65×10^4	4.42×10^{-2}	1.67×10^{-6}	5,144	26	76
Sulfo-NHS	2.52×10^4	4.45×10^{-2}	1.76×10^{-6}	6,111	38	93

A. ProteOn GLH chip



B. Multiuser SPR study



Analytical response of CBS binding versus the amount of CA II immobilized onto the sensor chip. A, ProteOn GLH chip; **B**, conventional chip (Myszka et al. 2003). The black dotted line shows the theoretical maximal response, assuming that 100% of the bound ligand molecules are active. The gold line is a linear fit of the actual response values. Actual ligand activity is 82% of theoretical for the sulfo-NHS activated GLH chip and 46% for the conventional NHS activated chip surfaces.

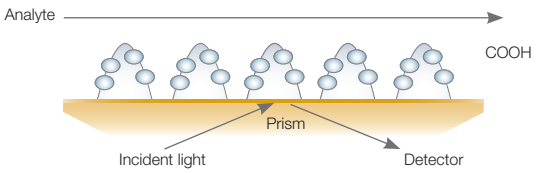
ProteOn Sensor Chips Expand Your SPR Applications

Three ProteOn sensor chips are available for general amine coupling. The GLC, GLM, and GLH chips provide for low, medium, and high ligand surface capacities, respectively. These three chips are functionalized with easily activated carboxylic acid groups, which can be activated by reagents such as EDC and sulfo-NHS to react specifically with free surface amines of proteins.

GLC Sensor Chip — Compact Capacity

The GLC sensor chip features a thin alginate layer for amine coupling of ligands at a compact (~6 kRU) surface capacity. The nearly planar surface layer of the GLC chip helps mitigate mass transport effects that are often observed with thicker surface layers. This versatile sensor chip is ideal for probing protein-protein interactions.

A



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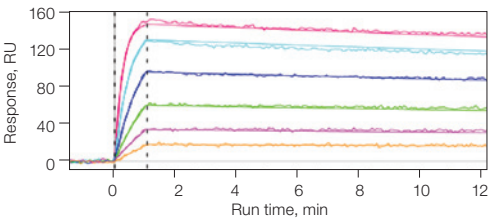

ProteOn GLC sensor chip. A, The thin alginate coating on the GLC sensor chip is responsible for creating a compact capacity surface. **B**, Sensorgrams of the cytokine IL-2 and an anti–IL-2 antibody interaction using the GLC sensor chip. The IL-2 Ab was immobilized to approximately 2,000 RU, and IL-2 was injected in a twofold dilution series ranging from 80 nM to 2.5 nM. The nearly planar surface of the GLC sensor chip allows for high-quality kinetic analysis.

GLM Sensor Chip — Medium Capacity

The GLM sensor chip is coated with a thicker alginate layer that displays an increased number of carboxylic acid groups for amine coupling of ligands at a medium capacity (~12 kRU). It can be used for both protein-protein interactions and protein–small molecule interactions.

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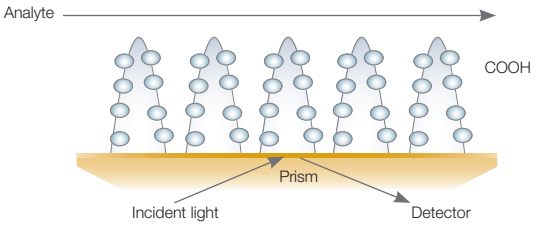

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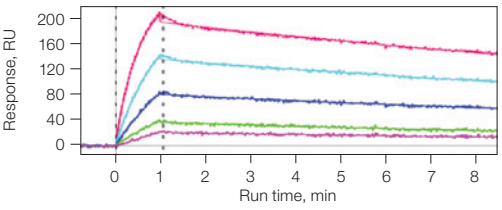
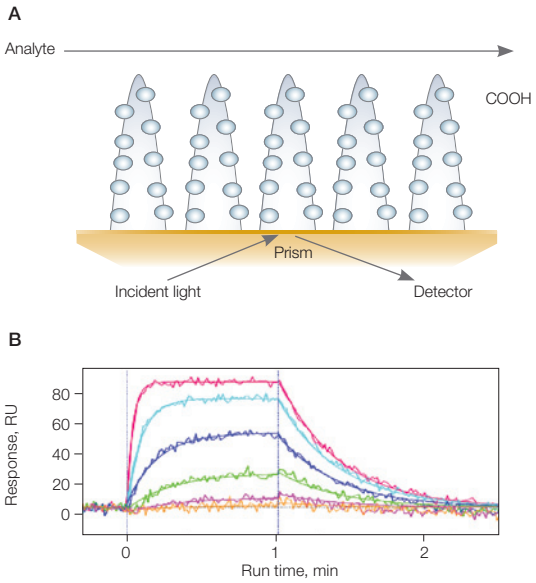


ProteOn GLM sensor chip. A, The extended alginate coating on the GLM sensor chip responsible for creating a medium-capacity surface. **B**, Sensorgrams of a TEM1 β-lactamase mutant interacting with the β-lactamase inhibitor protein (BLIP) using the GLM sensor chip. TEM1 was immobilized to approximately 1,500 RU, and BLIP was injected in a twofold dilution series ranging from 600 nM to 38 nM.

GLH Sensor Chip — High Capacity

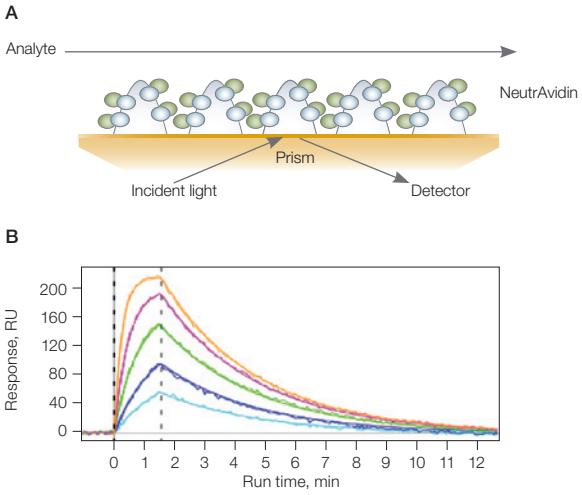
The GLH sensor chip is designed with a high-density alginate layer that contains an even greater number of carboxylic acid groups for amine coupling of ligands at a high (~20 kRU) surface capacity. This dense alginate layer on the GLH sensor chip is far superior at binding high-capacity ligand surfaces when compared to ligand immobilization results using the GLC and GLM sensor chips. This sensor chip is ideal for analyzing protein–small molecule (<1 kD) interactions, as the high-capacity surface gives an increased binding response. A comparison between the GLH sensor chip and a competitor's high-capacity sensor chip shows the full advantage of the easily activated carboxylic groups on the GLH sensor chip: significantly higher binding capacity and activity, leading to a much higher analyte response.



ProteOn GLH sensor chip. A, The dense alginate coating on the GLH sensor chip responsible for creating a high surface capacity. **B**, Sensorgrams of the interaction between carbonic anhydrase II (30 kD) and the inhibitor 4-carboxybenzenesulfonamide (CBS) (201 dalton) using the GLH sensor chip. Carbonic anhydrase II was immobilized to approximately 24,000 RU, and CBS was injected in a threefold dilution series ranging from 20 µM to 0.082 µM.

NLC Sensor Chip

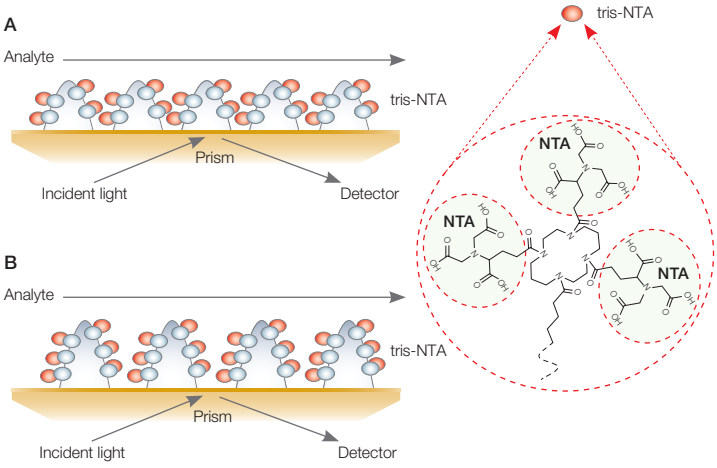
The NLC sensor chip has NeutrAvidin bound to the alginate layer and is used for capturing biotinylated proteins, peptides, and nucleic acids. It has the capacity to capture ~2,000 RU of IgG or ~500 RU of DNA. The NLC sensor chip is ideal for immobilizing ligands without amine coupling but requires that the ligand be modified with biotin prior to immobilization.



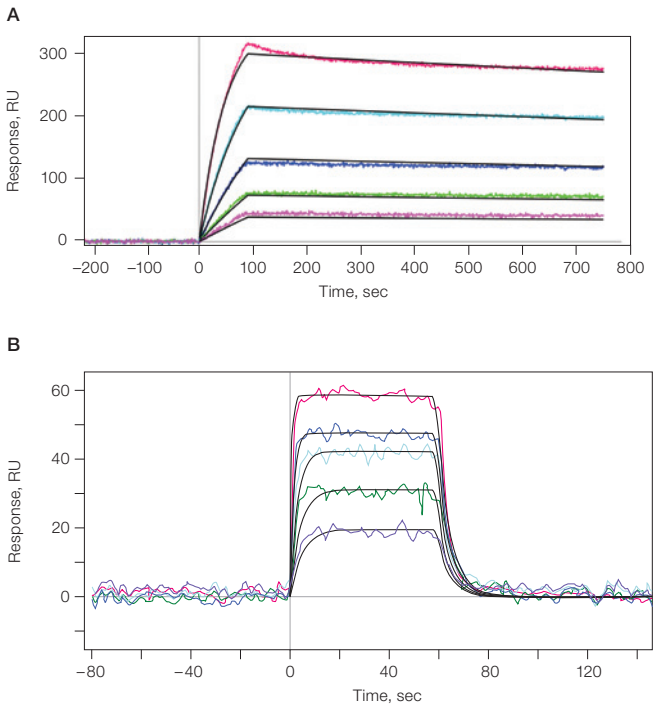
ProteOn NLC sensor chip. A, The NeutrAvidin-modified alginate coating on the NLC sensor chip. **B**, Sensorgrams of the interaction between an antibody Fab fragment and biotinylated MHC I/Tyr antigen using the NLC sensor chip. MHC I was captured to approximately 800 RU, and the Fab was injected in a twofold dilution series ranging from 500 nM to 31 nM.

HTG and HTE Sensor Chips

The HTG and HTE sensor chips feature a novel tris-NTA (3 x NTA) surface for improved capturing of histidine-tagged proteins. This tris-NTA functional group is unique to the ProteOn HTG and HTE sensor chips and has a significantly higher binding stability compared to the traditional mono-NTA (NTA) surface. This results in minimal ligand drift and improves sensorgram baseline stability. Bio-Rad offers two ProteOn sensor chips for various histidine-tagged protein applications: HTG for compact density or large molecule applications, and HTE for high-density or small molecule applications. Both the HTG and HTE sensor chips allow easy surface regeneration, chip reuse, and capture of histidine-tagged proteins directly from crude samples.



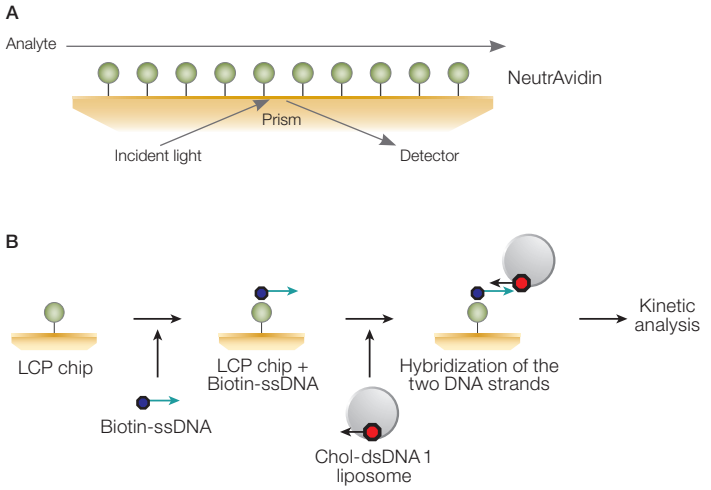
ProteOn HTG and HTE sensor chips. A, Alginate coating modified with compact-density tris-NTA on the HTG sensor chip; **B**, Alginate coating modified with high-density tris-NTA on the HTE sensor chip.



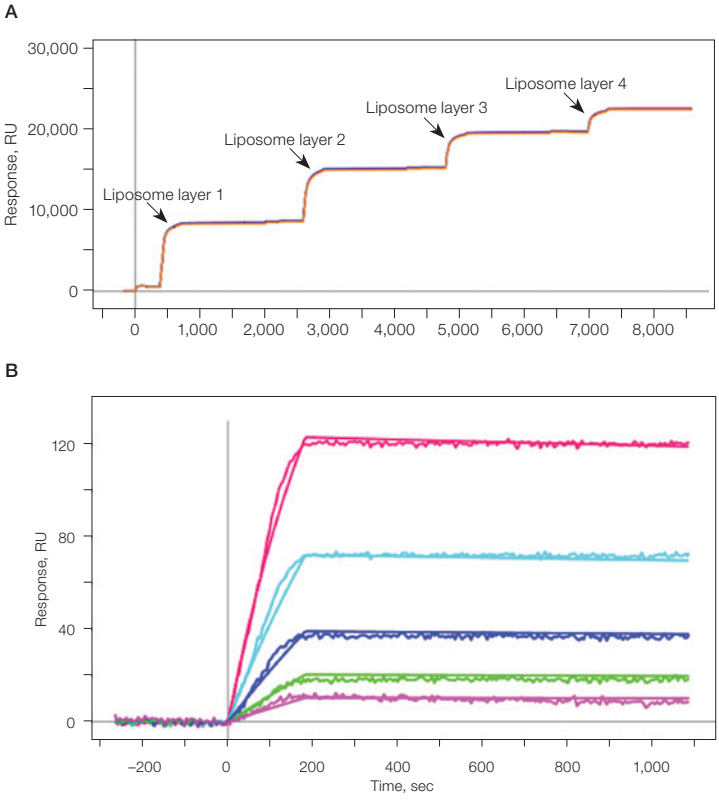
ProteOn HTG and HTE sensor chips. A, Sensorgrams of the interaction between the histidine-tagged protein A and IgG, showing the ability of the HTG chip to resolve high-affinity kinetics requiring long dissociation times. Protein A was captured to approximately 60 RU, and human IgG was injected in a twofold dilution series ranging from 100 nM to 6.3 nM. **B**, Sensorgrams of the interaction between histidine-tagged ERK2 (a MAP kinase) and the inhibitor Purvalanol B (432.9 dalton), showing that small molecules can be screened using the HTE chip. ERK2 was captured to approximately 12,800 RU, and Purvalanol B was injected in a threefold dilution series ranging from 50 µM to 0.62 µM.

LCP Sensor Chip

The LCP sensor chip provides a surface functionalized with NeutrAvidin in a planar configuration that is formed as a self-assembled monolayer. It is designed for use with the ProteOn LCP capturing reagent kit for lipid-protein, lipid–small molecule, and membrane protein-protein interaction analysis. The reagent kit activates the chip surface by a biotinylated DNA tag so that the chip is able to capture DNA-labeled lipid assemblies through DNA hybridization. The reagent kit is able to attach DNA tags to the lipid assemblies in order to anchor them to the chip surface. It is possible to capture two or more layers of lipid assemblies for additional sensitivity. This method allows for lipid-based interaction analysis, including the analysis of membrane proteins embedded in a lipid bilayer.



ProteOn LCP sensor chip. A, Planar NeutrAvidin-modified self-assembled monolayer on the LCP sensor chip. **B**, Workflow for liposome capturing using the LCP sensor chip and the LCP capturing reagent kit. Chol-dsDNA 1 and biotin-ssDNA contain complementary DNA sequences. The LCP sensor chip surface is saturated with single-stranded biotinylated DNA molecules, and then liposomes incubated with chol-dsDNA 1 are captured to the surface through DNA hybridization. For reagents and techniques used in this workflow, refer to bulletin 6161.



ProteOn LCP sensor chip. A, The planar NeutrAvidin surface and the LCP capturing reagent kit allow stable capturing of several POPC liposome layers. **B**, Detailed kinetic analysis of the interaction between FITC-labeled DSPC liposomes captured on the LCP sensor chip and an anti-FITC antibody. FITC-labeled DSPC liposomes were captured to approximately 330 RU, and the anti-FITC antibody was injected in a twofold dilution series ranging from 10 nM to 0.63 nM.

Ordering Information

Catalog #	Description
Sensor Chips	
176-5011	**ProteOn GLC Sensor Chip**, for general amine coupling, compact polymer layer with binding capacity of approximately one protein monolayer
176-5012	**ProteOn GLM Sensor Chip**, for general amine coupling, polymer matrix layer with intermediate binding capacity
176-5013	**ProteOn GLH Sensor Chip**, for general amine coupling, polymer matrix layer with highest binding capacity
176-5021	**ProteOn NLC Sensor Chip**, for binding of biotinylated molecules, contains NeutrAvidin immobilized to GLC layer
176-5031	**ProteOn HTG Sensor Chip**, for capturing of histidine-tagged proteins, polymer matrix layer contains tris-NTA complexes with compact binding capacity
176-5033	**ProteOn HTE Sensor Chip**, for capturing of histidine-tagged proteins, polymer matrix layer contains tris-NTA complexes with higher binding capacity
176-5041	**ProteOn LCP Sensor Chip**, for capturing lipid assemblies such as liposomes, for use with the ProteOn LCP capturing reagent kit
Sensor Chip Application Kits	
176-2300	**ProteOn Liposome Capturing Kit**, includes 1 ProteOn LCP sensor chip, 1 ProteOn LCP capturing reagent kit, and ProteOn lipid modification conditioning solution
176-2350	**ProteOn GLC Lipid Kit**, includes 1 ProteOn GLC sensor chip and 1 ProteOn lipid modification kit
176-2500	**ProteOn HTG Capturing Kit**, includes 1 ProteOn HTG sensor chip and 1 ProteOn HTG and HTE reagent kit
176-2600	**ProteOn HTE Capturing Kit**, includes 1 ProteOn HTE sensor chip and 1 ProteOn HTG and HTE reagent kit

Catalog #	Description
Reagent Kits	
176-2110	**ProteOn Immobilization Buffer Kit**, includes 1 each sodium acetate buffer (pH 4.0, 4.5, 5.0, 5.5)
176-2210	**ProteOn Regeneration and Conditioning Kit**, includes 1 each glycine buffer (pH 1.5, 2.0, 2.5, 3.0), and NaOH, SDS, HCl, phosphoric acid, NaCl; 50 ml each solution
176-2310	**ProteOn LCP Capturing Reagent Kit**, for capturing lipid assemblies such as liposomes, for use with the ProteOn LCP sensor chip
176-2360	**ProteOn Lipid Modification Kit**, includes ProteOn lipid modification conditioning solution and ProteOn lipid modification solution
176-2410	**ProteOn Amine Coupling Kit**, includes EDAC (EDC), sulfo-NHS and ethanolamine HCl
176-2510	**ProteOn HTG and HTE Reagent Kit**, includes reagents for activation and regeneration of HTG and HTE sensor chips

References

Myszka DG et al. (2003). The ABRF-MIRG'02 study: assembly state, thermodynamic, and kinetic analysis of an enzyme/inhibitor interaction. J Biomol Tech 14, 247–269.

For more information on the ProteOn XPR36 protein interaction array system, visit **www.bio-rad.com/proteon**.

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