



## 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

## Application-Specific Surface Chemistries = Optimized Ligand Activity

### 6 x 6 — A Powerful Equation

Used with the ProteOn XPR36 protein interaction array system, ProteOn sensor chips combine advanced surface chemistries with state-of-the-art microfluidics. Robust, real-time, label-free biomolecular interaction data are generated in the 6 x 6 sensor chip interaction array. With ProteOn sensor chips, up to 36 unique biomolecular interactions can be assayed simultaneously in one experiment, providing valuable kinetic, equilibrium, and concentration data. Turning the research work of days into hours, ProteOn sensor chips are ideal for accelerated protocol development, One-shot Kinetics™ analysis, multiple protein interaction analysis, and high-throughput screening of multiple samples. Each ProteOn sensor chip is enclosed in a bar-coded cartridge for reliable data tracking and archiving. ProteOn sensor chips provide flexible use of all six ligand channels across different experiments.



### ProteOn Sensor Chip Performance

ProteOn sensor chips\* are designed and have been thoroughly tested for reliable real-time measurement of multiple protein-protein, protein-peptide, protein-small molecule, and protein-DNA interactions. ProteOn sensor chips provide:

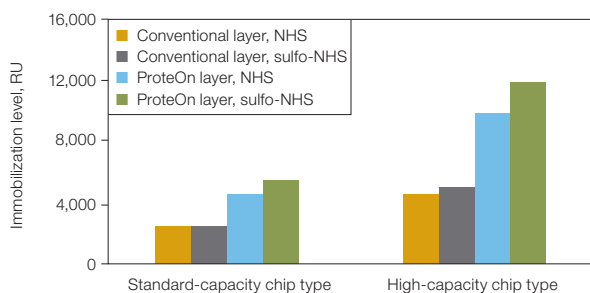
- High binding capacity
- Outstanding kinetic response
- Sufficient sensitivity for detection of low-molecular weight (<95 Da) analytes
- Uniform spot-to-spot response
- Long-term storage stability

\* ProteOn sensor chips are packaged with an inert gas in a sealed pouch. Stored properly at 4°C, the shelf life is 2 years from date of production. Chips are guaranteed to have at least 6 months of shelf life upon receipt.

**BIO-RAD**

## 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 easily activated layers is higher than conventional layers, and activation of ProteOn layers is higher using sulfo-NHS instead of NHS.

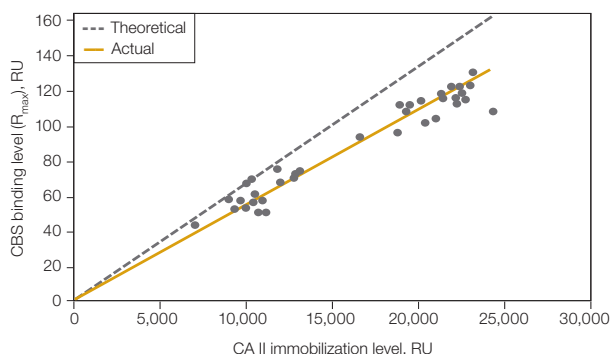
### Representative immobilization efficiencies on ProteOn sensor chip surfaces designed for high protein binding capacity.

Protein	pI	Non-Bio-Rad	GLM Chip,	GLM Chip,	GLH Chip,
		Chip, NHS	NHS	Sulfo-NHS	Sulfo-NHS
		Activation, RU	Activation, RU	Activation, RU	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
$\beta$ 2-microglobulin	5.3	2,600	3,250	3,650	12,400
Carbonic anhydrase II	5.9	6,600 $\pm$ 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

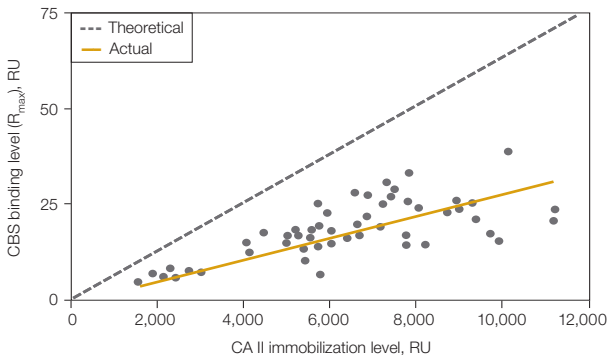
**Comparative binding data for TEM1 KSSR/BLIP and carbonic anhydrase II/CBS interactions.** The use of sulfo-NHS instead of NHS contributes to higher ligand binding, ligand activity, and analyte signals.

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

### A. ProteOn GLH chip



### B. Multiuser SPR study

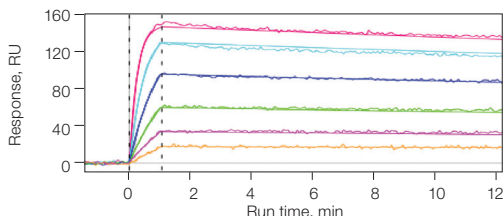


**Analytical response of CBS binding versus the amount of CAII immobilized onto the sensor chip.** A, ProteOn GLH chip; B, conventional chip (Myszka et al. 2003). The black dotted line shows the theoretical maximal response, assuming that 100% of the bound ligand molecules are active. The gold line is 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.

## Five ProteOn Sensor Chips for Numerous Research Applications

### ProteOn GLC Sensor Chip

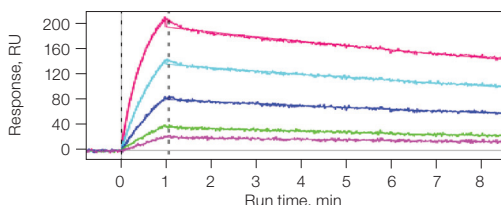
The ProteOn GLC sensor chip has active carboxyl groups for covalent immobilization of biomolecules via amine groups. This versatile sensor chip is designed to bind a monolayer of analyte and is ideal for protein-protein interaction analysis.



**ProteOn GLC sensor chip.** Monolayer surface provides detailed kinetic analysis of the cytokine IL-2/IL-2 antibody interaction.

### ProteOn GLM Sensor Chip

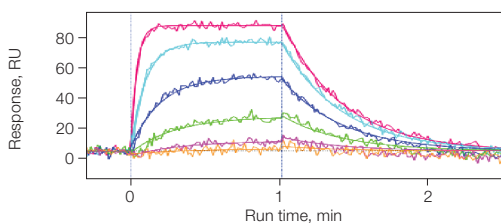
The ProteOn GLM sensor chip has active carboxyl groups for covalent immobilization of biomolecules via primary amine groups. This versatile sensor chip displays a rough carboxyl surface layer to provide higher ligand binding capacity for protein-protein and some small molecule interactions.



**ProteOn GLM sensor chip.** Medium-capacity polymer surface provides detailed kinetic analysis of the interaction between TEM mutant S130/S235 and wild type BLIP.

### ProteOn GLH Sensor Chip

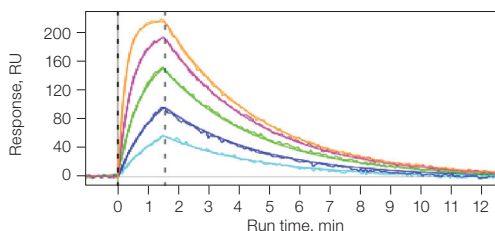
The ProteOn GLH sensor chip also has active carboxyl groups for covalent immobilization via primary amine groups. This chip consists of a highly extended polymer layer for maximum binding capacity and is ideal for protein-small molecule and more difficult interactions.



**ProteOn GLH sensor chip.** Highest-capacity polymer surface provides detailed kinetic analysis of the interaction between carbonic anhydrase II (30 kD) and 4-carboxybenzenesulfonamide (CBS) (MW 201).

### ProteOn NLC Sensor Chip

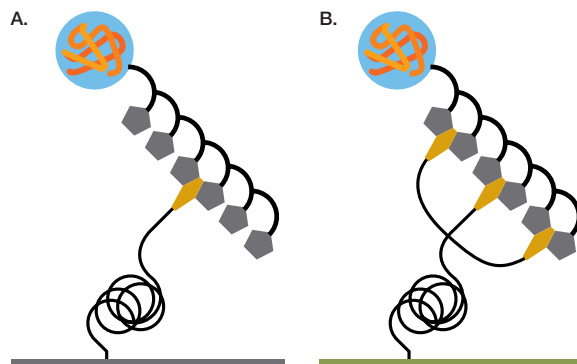
The ProteOn NLC sensor chip features NeutrAvidin bound to the polymer layer and is ideal for immobilization of biotinylated proteins, peptides, and nucleic acids.



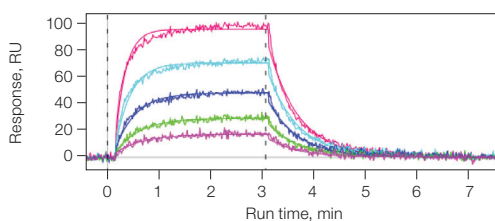
**ProteOn NLC sensor chip.** NeutrAvidin-bound surface provides detailed kinetic analysis of the interaction between antibody Fab fragment and biotinylated MHC I/Tyr antigen.

### ProteOn HTG Sensor Chip

The ProteOn HTG sensor chip features a novel tris-NTA complex for improved capturing of His-tagged proteins. The HTG sensor chip properties allow surface regeneration for chip re-use and the capturing of His-tagged proteins directly from crude cell lysates. This His-tag sensor chip is an ideal choice for protein-protein and protein-peptide interactions.



**Comparison of mono-NTA and tris-NTA binding to His-tagged proteins.** **A**, Mono-NTA is the traditional method for capturing His-tagged proteins but achieves weak binding and results in ligand drift, unstable baselines, distorted kinetic results, and inaccurate fitting to a kinetic model. **B**, ProteOn trivalent tris-NTA complexes improve binding stability and increase binding selectivity to His-tagged molecules. Ni-NTA (●) binding to histidine (●) occurs after activation with  $\text{Ni}^{2+}$ .



**ProteOn HTG sensor chip.** Tris-NTA surface provides detailed kinetic analysis of the interaction between His-tagged IFNAR1 and interferon YNS mutant.

## Reference

Myszka et al. (2003). Capture and reconstitution of G protein-coupled receptors on a biosensor surface. *Anal Biochem* 316, 243–250.

## Ordering Information

Catalog #	Description
176-5011	<b>ProteOn GLC Sensor Chip</b> , for general amine coupling, compact polymer layer with binding capacity of approximately one protein monolayer
176-5012	<b>ProteOn GLM Sensor Chip</b> , for general amine coupling, polymer matrix layer with intermediate binding capacity
176-5013	<b>ProteOn GLH Sensor Chip</b> , for general amine coupling, polymer matrix layer with highest binding capacity
176-5021	<b>ProteOn NLC Sensor Chip</b> , for binding of biotinylated molecules, contains NeutrAvidin immobilized to GLC layer
176-5031	<b>ProteOn HTG Sensor Chip</b> , for capturing of His-tagged proteins, polymer matrix layer contains tris-NTA complexes

For more information on the ProteOn XPR36 protein interaction array system, visit [www.bio-rad.com/proteon](http://www.bio-rad.com/proteon).

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