Protein Interaction Analysis

ProteOn™ XPR36
Protein Interaction Array System

The Power of Parallel Analysis
The ProteOn XPR36 protein interaction array system is a surface plasmon resonance (SPR) biosensor platform that provides real-time label-free analysis of the specificity, affinity, and kinetics of biomolecular interactions. Using the XPR36 configuration, this system generates a 6 x 6 interaction array for the simultaneous analysis of up to six ligands with up to six analytes. The ProteOn XPR36 system increases the versatility of experiment design and the productivity of experimental workflow, enabling the completion of high-quality SPR experiments very efficiently. The parallel-flow SPR biosensor platform:

- Analyzes up to 36 different protein interactions in a single run on a single chip
- Measures a variety of experimental conditions simultaneously using parallel-flow fluidics
- Screens multiple panels of analytes
- Acquires the resonance angle shift as SPR response units (RU) for accurate kinetics
- Employs One-shot Kinetics™ technology, which enables a complete kinetic analysis in a single run

Biomolecular Interaction in a New Light

Two biomolecules, A and B, interact with each other to form a complex AB. Using an SPR biosensor, besides the equilibrium constant $K_D$, the association rate constant $k_a$ and the dissociation rate constant $k_d$ can be measured, determining more details of the interaction compared to other methods.
ProteOn XPR36 System

Key Applications in Research and Discovery

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<tr>
<th>Basic Research</th>
<th>Applied Research</th>
<th>Screening</th>
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<td>- Identification of biomolecular interactions</td>
<td>- Identification and validation of drug targets</td>
<td>- Monoclonal antibody screening</td>
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<tr>
<td>- Characterization of biomolecular structures</td>
<td>- Rapid assay design</td>
<td>- Small molecule and fragment screening</td>
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</table>

Lead Optimization

- Antibody epitope binning
- Antibody epitope mapping
- Kinetic and thermodynamic characterization of drug-target interactions

Preclinical/Clinical

- Serum sample analysis
- Vaccine validation
- Clinical assay design

Process/Quality Control

- Active concentration analysis
- Quantitation and kinetics in 1 workflow
- 21 CFR Part 11 compliance

ProteOn XPR36 System Advantages

Versatility

- Flexible experimental configuration
- Efficient experimental optimization
- Kinetic, affinity, and thermodynamic analysis in 1 platform
- Compatible with crude samples
- Compatible with both qualitative and quantitative assays
- Compatible with direct and indirect binding assays

Productivity

- Analysis of 36 interactions in a single injection
- Up to 6 full kinetics measured in a single injection in 1.1 hr
- Full kinetics of 96 antibody supernatant samples in 11 hr
- Unattended running by system automation
- Batch data processing and analysis by software

High Quality

- Label-free analysis with native protein structures
- Novel referencing options for accurate kinetics and affinity
- Excellent sensitivity
- Multiple surface chemistries for optimal assay conditions
- Parallel-flow fluidics allowing for real-time comparison of multiple interactions

Biomolecular interaction analysis does not only mean measuring the binding affinity. The ProteOn XPR36 system characterizes the following aspects of a biomolecular interaction:

- How specific is the interaction? [Kd]
- How fast is the interaction [ka]?
- How stable is the complex [kd]?
- How strong is the interaction [KD = kd / ka]

The parameters are obtained from the data fitting of the association, equilibrium (optional), and dissociation phases of a sensorgram.

![SPR sensorgram](image)
## ProteOn XPR36 System Applications

### Antibody Characterization and Profiling
Screen antibody-antigen interactions, including kinetics, epitope mapping, and epitope binning.

- Enzyme-linked immunosorbent assay (ELISA)
- Isothermal calorimetry (ITC)
- Conventional serial flow SPR

### Drug Discovery Screening
Analyse protein–small molecule interactions, such as screening compounds for drug discovery.

- Competitive ELISA
- ITC
- Conventional serial flow SPR

### Protein-Protein Interactions
Analyse protein-protein interactions to pinpoint structures on proteins that are responsible for binding.

- X-ray crystallography
- ITC
- Conventional serial flow SPR

### Protein Quantitation and Kinetics
Analyze the active concentration of a protein sample by the initial binding rate.

- ELISA
- Biolayer interferometry
- Conventional serial flow SPR

### Advantages of ProteOn XPR36 System

#### Antibody Kinetic Screening
- Efficient experimental optimization
- Accurate kinetics
- Compatible with crude samples
- High-throughput screening

#### Epitope Mapping/Epitope Binning
- Flexible experimental configurations
- Available for various types of assays
- High-throughput screening

#### Traditional Methods
- High sensitivity
- High-throughput screening
- Available for fragment screening
- Accurate kinetics

#### Protein-Protein Interactions
- Efficient experimental optimization
- HTG and HTE sensor chips based on tris-NTA (3 x NTA) surface chemistry for stable and regenerable capture of histidine-tagged proteins
- Efficient online purification process using tris-NTA (3 x NTA) or other surface chemistries for antibody screening or mutagenesis in structural biology
- Compatible with crude samples
- Accurate kinetics

#### Protein Quantitation and Kinetics
- Accurate quantitation and kinetics
- Wide dynamic range in quantitation
- High throughput for rapid sample processing

### Assay Design
Discover the optimal design and experimental conditions for high-quality biological assays.
- ELISA
- Conventional serial flow SPR

### Lipid-Based Interactions
Analyze interactions of lipid bilayer membranes or membrane proteins with other biomolecules using SPR.
- Lipophilic surface chemistry

### Thermodynamics and Energetics
Analyze thermodynamics to further characterize biomolecular interactions.
- ITC
  - Conventional serial flow SPR

### Cell Surface Interactions
Analyze the interaction between a target and a cell, including bacterial and mammalian cells.
- Flow cytometry
- Label-free cell morphology sensing
- Quartz crystal microbalance
- Conventional serial flow SPR

- Rapid label-free screening for assay components and conditions
- Rapid epitope binning for designing sandwich immunoassays
- Versatile assay configurations
- Multiple surface chemistries for different types of assays
- Unattended running for assay validation
- ProteOn liposome capturing kit based on hydrophilic surface chemistry using DNA hybridization
- Hydrophilic surface chemistry for easy regeneration and high performance when capturing lipid assemblies
- ProteOn GLC lipid kit based on traditional lipophilic surface chemistry using alkyl modification; provides customized surface lipophilicity for optimal performance
- Real-time referencing for reliable experimental results
- Highly efficient thermodynamic analysis workflow for structural biology
- Experimental repeats in a single run for accurate thermodynamics and energetics
- Rapid label-free assays
- Efficient experimental optimization
- Flow channels compatible with cell samples to avoid clogging in the system
SPR—the Key Technology for Biomolecular Interaction Analysis

Interaction between biomolecules is of great interest in biological research. Understanding the whole set of biomolecular interactions in a cell, known as an interactome, lays the foundation of molecular and cell biology. There are different technologies available for biomolecular interaction analysis, which can be categorized as real-time analysis and end-point analysis.

- Real-time analysis technologies provide a complete time trace and kinetics of a biomolecular interaction
- End-point analysis technologies provide a readout after a biomolecular interaction takes place

**A. Slow Off-Rate Biomolecular Interaction**

Real-time vs. end-point analysis. SPR technology analyzes all types of biomolecular interactions, including those with slow and fast off-rates. **A**, slow off-rate biomolecular interactions, which typically occur with strong binding affinity, can be measured by both SPR and end-point analysis methods, such as communoprecipitation (CoIP)–western blot. In addition, SPR provides kinetics for further characterization. **B**, fast off-rate biomolecular interactions, which typically occur with weak binding affinity, can also be measured by SPR. However, these interactions are difficult to measure or even detect using end-point analysis methods because of their dissociation in rinsing steps. RU, response units.

**B. Fast Off-Rate Biomolecular Interaction**

Workflow Using the ProteOn XPR36 System for an SPR Experiment

1. Start up system
2. Load a sensor chip
3. Create a protocol
4. Load samples

Bio-Rad Laboratories, Inc.
Kinetic analysis. Different biomolecular interactions with the same affinity (K_D) may have diversified binding kinetics (k_a and k_d). RU, response units.

SPR technology is amenable to high-throughput platforms. The ProteOn XPR36 system features a 6 x 6 interaction array available for high-throughput applications.

SPR, as a key technology in this field providing real-time biomolecular interaction analysis in a label-free manner, offers unique benefits that are not available with other technologies.
ProteOn XPR36 Protein Interaction Array System

SPR-based biosensors determine binding kinetics of protein-protein interactions by measuring refractive index changes on an optical surface. The ProteOn XPR36 system is a parallel-flow SPR biosensor platform featuring 36 interaction spots on a 6 x 6 array.

Benefits of 6 x 6 Array

Versatility – Multiple Experimental Configurations and Fast Qualitative and Quantitative Assays

Kinetic Characterization (1-to-1)

In kinetic characterization experiments, the optimization of experimental protocols is usually the most labor-intensive and time-consuming step. Probing at one time six ligand immobilization conditions together with six analyte injection conditions, the ProteOn XPR36 system allows for full optimization in a single run. This ensures the optimal experimental conditions for the interaction between the ligand and the analyte. The method of using a single run of 6 x 6 injections for a complete kinetic analysis is called One-shot Kinetics.

Kinetic Screening (6-to-1)

In kinetic screening experiments, each of the six ligand channels gives a full kinetic analysis in a single run. This high throughput enables fast processing of a large number of samples while accurate kinetics is maintained. The ProteOn XPR36 system provides the best balance between throughput and accuracy of kinetic screening.

Multiplex Screening (6-to-6) and Array Screening (36-to-1)

In multiplex or array screening experiments, the 6 x 6 interaction array of the ProteOn XPR36 system is fully utilized for high throughput, which enables multiplex or 36-ligand screening.
Productivity – Fast Protocol Optimization and High Throughput

Kinetic Characterization
- ProteOn XPR36 system: 1.1 hr, 6 full kinetics (36 data points, surface regeneration not required)
- Conventional serial flow SPR system: 3.5 hr, 3 full kinetics (18 data points, surface regeneration required)

Kinetic Screening
- Captured Ligand Screening (for mAb supernatants)
  - ProteOn XPR36 system: 11 hr, 96 full kinetics (576 data points)
  - Conventional serial flow SPR system: 65 hr, 96 full kinetics (576 data points)
- Analyte Screening
  - ProteOn XPR36 system: 25 hr, 96 x 6 full kinetics (3,456 data points)
  - Conventional serial flow SPR system: 5.7 days, 96 x 3 full kinetics (1,728 data points)

Multiplex Screening
- ProteOn XPR36 system: 0.7 hr, 6 x 6 binning matrix (36 data points)
- Conventional serial flow SPR system: 2.5 hr, 3 x 6 binning matrix (18 data points)

Array Screening
- ProteOn XPR36 system: 12 hr, 36 x 36 binning matrix (1,296 data points)
- Conventional serial flow SPR system: no equivalent
Four Factors for High-Quality SPR Results

1. **SPR System**
   - **Sufficient Signal to Noise Ratio**
     - ProteOn XPR36 system signal-to-noise ratio. ProteOn XPR36 system noise is 1 RU and ~2 RU after double referencing. SPR responses over three times signal-to-noise ratio (3 x SNR) are detectable. RU, response units.

2. **Experiment Design**
   - **XPR36 Configuration Optimizes Multiple Factors Simultaneously**

3. **Data Processing**
   - **Data Referencing**
     - The key step in data processing is data referencing. Data referencing corrects for the artifacts in SPR experimental results.
     - The ProteOn XPR36 system has two novel advantageous referencing modes that no other SPR system provides: an interspot reference to correct for refractive index change (bulk effect) and nonspecific binding, and a real-time injection reference to correct for baseline drift resulting from the changes of the ligand surface.
     - **Note:** For additional information about the referencing options in the ProteOn XPR36 system, watch [www.bio-rad.com/proteon/reference](http://www.bio-rad.com/proteon/reference).

4. **Data Analysis**
   - **Software Advantages**
     - ProteOn Manager™ software is a comprehensive, user-friendly tool for the analysis of biomolecular interactions.
     - Ease of use
     - Integration of data acquisition, data processing, and data analysis
     - Powerful graphic user interface
     - Intuitive protocol writing interface
     - Fast and accurate data processing
     - Accurate fitting with 8 models
     - Rapid data analysis
     - Concise analysis reports
     - Export functions for further data processing in Excel or other software

**Note:** For additional information about the referencing options in the ProteOn XPR36 system, watch [www.bio-rad.com/proteon/reference](http://www.bio-rad.com/proteon/reference).
Surface Chemistries

Direct Coupling of Targets
The ProteOn GLC, GLM, and GLH sensor chips are designed for direct coupling of proteins and peptides, offering compact, medium, and high surface capacity levels, respectively.

ProteOn GLC sensor chip. The interaction analysis between cytokine IL2 and an anti-IL2 antibody was achieved using the compact-capacity GLC chip. RU, response units.

ProteOn GLM sensor chip. The interaction analysis between a TEM1 β-lactamase mutant and the β-lactamase inhibitor protein (BLIP) was achieved using the medium-capacity GLM chip. RU, response units.

ProteOn GLH sensor chip. The interaction analysis between carbonic anhydrase II and an inhibitor carboxybenzenesulfonamide (MW 201) was achieved using the high-capacity GLH chip. RU, response units.

Capturing of Targets
The ProteOn NLC sensor chip is designed for site-specific capturing of biotinylated biomolecules. ProteOn HTG and HTE sensor chips are designed for site-specific capturing of histidine-tagged proteins.

ProteOn NLC sensor chip. The interaction analysis between an antibody Fab fragment and biotinylated MHC I/Tyr was achieved using the NLC chip. RU, response units.

ProteOn HTG sensor chip. The interaction analysis between histidine-tagged protein A and human IgG was achieved using the HTG chip, showing its capability to resolve high-affinity kinetics requiring long dissociation time. RU, response units.

ProteOn HTE sensor chip. The interaction analysis between histidine-tagged ERK2 (a MAP kinase) and the inhibitor purvalanol B (MW 433) was achieved using the HTE chip, showing its capability to screen small molecules. RU, response units.

Capturing of Lipid Assemblies
The ProteOn LCP sensor chip together with the ProteOn LCP capturing reagent kit, and the ProteOn GLC sensor chip together with the ProteOn lipid modification kit are designed for capturing lipid assemblies for the analysis of lipid-bilayer membranes or membrane proteins.

ProteOn LCP sensor chip. The interaction analysis between FITC-labeled DSPC liposomes captured on the LCP sensor chip and an anti-FITC antibody was achieved using the LCP chip. RU, response units.

Modified ProteOn GLC sensor chip. The interaction analysis between POPC liposome and a small molecule drug, tetracaine (MW 264), was achieved using the modified GLC chip. RU, response units.
### Hardware Specifications

<table>
<thead>
<tr>
<th>Number of interaction spots</th>
<th>36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment temperature range</td>
<td>15–40°C</td>
</tr>
<tr>
<td>Dynamic range</td>
<td>1–40,000 RU absolute for all types of sensor chips, regardless of surface chemistry</td>
</tr>
<tr>
<td>Uniformity of response</td>
<td>&gt;98% (CV &lt; 2%)</td>
</tr>
<tr>
<td>Baseline drift</td>
<td>&lt;1 RU/min</td>
</tr>
<tr>
<td>Baseline noise</td>
<td>&lt;1 RU</td>
</tr>
<tr>
<td>Sample flow rate</td>
<td>25–200 µl/min</td>
</tr>
<tr>
<td>Sample flow rate uniformity in 6 parallel channels</td>
<td>&gt;99% (CV &lt; 1%)</td>
</tr>
<tr>
<td>Autosampler</td>
<td>Temperature-controlled sample rack for stable flow rate set operated in unison for uniform and stable flow rate</td>
</tr>
<tr>
<td>Syringe pumps</td>
<td>6 sample and 6 buffer syringes with each set operated in unison for uniform and stable flow rate</td>
</tr>
<tr>
<td>Sensor chip detection</td>
<td>Automatic bar code recognition of sensor chip type, expiration date, and previous experiment</td>
</tr>
</tbody>
</table>

### Software

- **PC operating system**: Windows XP or Windows 7
- **Program**: ProteOn Manager software
- **Data-fitting models**: Langmuir, Langmuir off-rate analysis, Langmuir with mass transfer, heterogeneous analyte, bivalent analyte, heterogeneous ligand, two states, Langmuir with drift
- **GXP (optional)**: 21 CFR Part 11; IQ/OQ software tools

### Typical Working Ranges

- **Molecular mass detection limit**: Typically >95 Da
- **Sample concentration**: Typically >10 µM
- **Association rate constant (k_{a})**: Typically 3 x 10^{−3}–3 x 10^{4} M/sec^{−1}
- **Dissociation rate constant (k_{d})**: Typically 1 x 10^{−6}–6 x 10^{−1} sec^{−1}
- **Equilibrium constant (K_{d})**: Typically 2 x 10^{−4}–1 x 10^{−15} M

### Ordering Information

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>176-0210</td>
<td>ProteOn Manager Software, 1-user license, includes 1 HASP key</td>
</tr>
<tr>
<td>176-0200</td>
<td>ProteOn Manager Software, 1-user license, includes 1 HASP key</td>
</tr>
</tbody>
</table>

### Sensor Chips

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Description</th>
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<tbody>
<tr>
<td>176-5011</td>
<td>ProteOn GLC Sensor Chip, for general amine coupling, compact polymer matrix layer with binding capacity of approximately 1 protein monolayer</td>
</tr>
<tr>
<td>176-5012</td>
<td>ProteOn GLM Sensor Chip, for general amine coupling, polymer matrix layer with intermediate binding capacity</td>
</tr>
<tr>
<td>176-5013</td>
<td>ProteOn GLH Sensor Chip, for general amine coupling, polymer matrix layer with highest binding capacity</td>
</tr>
<tr>
<td>176-5021</td>
<td>ProteOn NLC Sensor Chip, for binding of biotinylated molecules, contains NeutrAvidin immobilized to GLC layer</td>
</tr>
<tr>
<td>176-5031</td>
<td>ProteOn HTG Sensor Chip, for capturing histidine-tagged proteins, polymer matrix layer contains tri-NTA complexes with compact binding capacity</td>
</tr>
<tr>
<td>176-5033</td>
<td>ProteOn HTE Sensor Chip, for capturing histidine-tagged proteins, polymer matrix layer contains tri-NTA complexes with higher binding capacity</td>
</tr>
<tr>
<td>176-5041</td>
<td>ProteOn LCP Sensor Chip, for capturing lipid assemblies such as liposomes, for use with ProteOn LCP capturing reagent kit</td>
</tr>
</tbody>
</table>

### Sensor Chip Application Kits

| 176-2300  | ProteOn Liposome Capturing Kit, includes 1 ProteOn LCP sensor chip, 1 ProteOn LCP capturing reagent kit, and ProteOn LCP lipid modification conditioning solution |
| 176-2350  | ProteOn GLC Lipid Kit, includes 1 ProteOn GLC sensor chip and 1 ProteOn GLC 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 |

### Reagent Kits

- **ProteOn Immobilization Buffer Kit**, includes 1 each sodium acetate buffer (pH 4.0, 4.5, 5.0, 5.5)
- **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 solution each
- **ProteOn LCP Capturing Reagent Kit**, for capturing lipid assemblies such as liposomes, for use with ProteOn LCP sensor chip
- **ProteOn Lipid Modification Kit**, includes ProteOn lipid modification conditioning solution and ProteOn lipid modification solution
- **ProteOn Amine Coupling Kit**, includes EDAC (EDC), sulfo-NHS, and ethanamine HCl
- **ProteOn HTG and HTE Reagent Kit**, includes reagents for activation and regeneration of HTG and HTE sensor chips

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