PROTEIN INTERACTION ANALYSIS

ProteOn™ Protocol Development Kits

- Designed for both new and experienced users of the ProteOn XPR36 system
- Gain familiarity with experimental design and instrument operation
- Use as a benchmark for developing new protocols

Explore the Power of the ProteOn 6 x 6 Interaction Array

Benefits

The ProteOn protocol development kits provide the reagents and sensor chips needed to perform and analyze a complete experiment using the ProteOn XPR36 protein interaction array system. New users will gain familiarity with instrument setup and operation, experimental design, and data analysis. Experienced users will find the kits valuable for system benchmarking and as positive control reagents for protocol development. Each kit contains sufficient materials for a complete exercise in the operation of the ProteOn XPR36 system.

Three Protocol Development Kits

One-shot Kinetics™ kit — The interaction between the cytokine IL-2 and an IL-2 antibody demonstrates a detailed kinetic analysis in a single injection cycle without regeneration (One-shot Kinetics). The kit also demonstrates a useful method for controlling ligand immobilization levels during protocol development and optimization.

Multiple protein interaction kit — The interaction between TEM1 β-lactamase and b-lactamase inhibitor protein (BLIP) demonstrates the power of the ProteOn XPR36 system to produce a detailed kinetic analysis of multiple simultaneous interactions and to map protein interfaces.

Protein-small molecule kit — The interaction between carbonic anhydrase (CA) II and 4-carboxybenzenesulfonamide (CBS) demonstrates the capability of the ProteOn XPR36 system to detect low molecular weight analytes. The kit also provides an additional benchmark of optimal system performanc

BIO-RAD

ProteOn One-shot Kinetics Kit

The ProteOn One-shot Kinetics kit demonstrates a complete, detailed kinetic analysis of a protein-protein interaction in a single injection cycle.

This kit also demonstrates the utility of the ProteOn XPR36 system for protocol optimization. Ligand is immobilized to different levels in five parallel channels of a sensor chip, followed by a single injection cycle of an analyte concentration series in the six orthogonal channels (Figures 1 and 2). The third ligand channel is used as a reference channel. A set of sensorgrams providing detailed kinetic analysis data is produced from each interaction channel (Figure 3). Optimal immobilization conditions may thus be rapidly and efficiently determined using the parallel processing capability of the ProteOn XPR36 system. (For details, request bulletin 3172.)

The ProteOn One-shot Kinetics kit includes:

- IL-2/IL-2 antibody pair, with sufficient material to immobilize IL-2 antibody in up to 5 channels and to analyze a complete concentration series of IL-2 across each channel
- GLC sensor chip
- Amine coupling kit
- Acetate buffer, 10 mM, pH 4.5
- Instruction manual

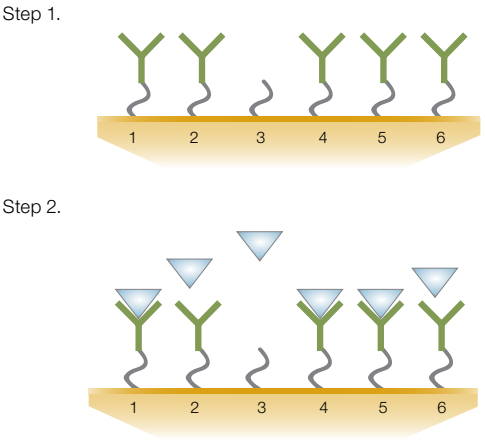

Fig. 1. Workflow of the ProteOn one-shot kinetics kit. Step 1, immobilize IL-2 antibody on chip surface. Step 2, inject IL-2 antigen and monitor interaction with immobilized IL-2 antibody. (Channel 3 is a reference channel.)

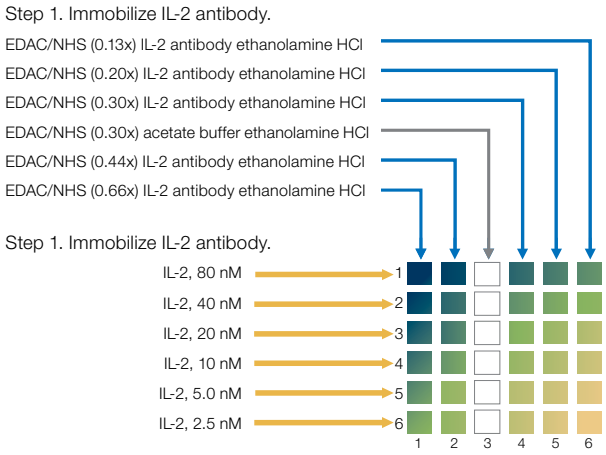

Fig. 2. Protocol optimization using the ProteOn One-shot Kinetics kit. Acetate buffer was at 10 mM, pH 4.5.

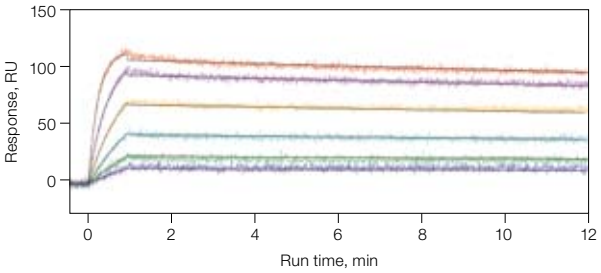

Fig. 3. Detailed kinetic analysis of the IL-2/IL-2 antibody interaction. Results obtained using the ProteOn One-shot Kinetics kit (—, 80 nM; —, 40 nM; —, 20 nM; —, 10 nM; —, 5 nM; —, 2.5 nM).

ProteOn Multiple Protein Interaction Kit

The ProteOn multiple protein interaction kit demonstrates the ProteOn XPR36 system's capability to analyze multiple protein-protein interactions simultaneously.

The kit contains five different mutant proteins of TEM1 and BLIP. Detailed kinetic data are obtained on the interaction of BLIP with each of the five TEM1 mutant proteins in a single injection cycle. The five TEM1 mutant proteins are immobilized in five of the six parallel channels on the sensor chip, and a BLIP concentration series is then injected into the six orthogonal channels (Figures 4 and 5). The third ligand channel is used as a reference channel. Five sets of six sensorgrams are simultaneously produced for rapid analysis and comparison of the binding kinetics of each TEM1 mutant protein with BLIP (Figure 6). (For details, request bulletins 5358 and 5368.)

The ProteOn multiple protein interaction kit includes:

- TEM1/BLIP protein set, with sufficient material to immobilize 5 TEM1 mutant proteins in 5 channels and to perform a detailed kinetic analysis of the interaction of each of the mutant proteins with BLIP
- GLC sensor chip
- Amine coupling kit
- Acetate buffer, 10 mM, pH 4.0
- Instruction manual

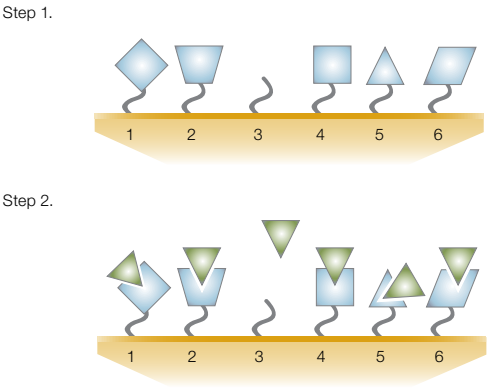

Fig. 4. Workflow of the ProteOn multiple protein interaction kit. Step 1, immobilize five TEM1 mutant proteins on five channels. Step 2, inject BLIP analyte proteins and monitor interaction with immobilized TEM1 proteins. (Channel 3 is a reference channel.)

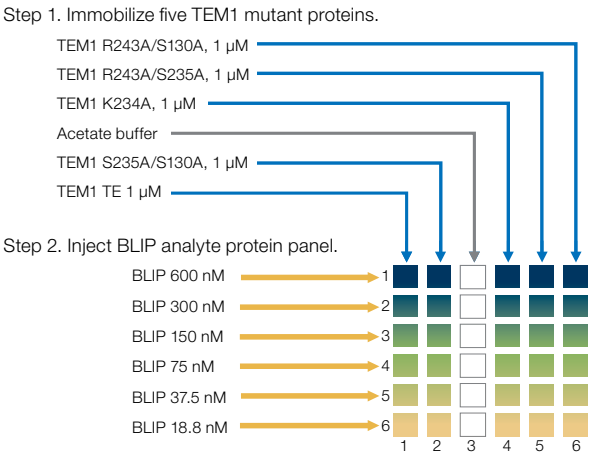

Fig. 5. One-shot Kinetic analysis of the TEM1/BLIP interaction. Acetate buffer was at 10 mM, pH 4.0.

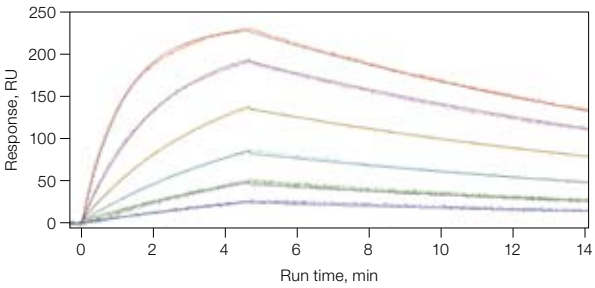

Fig. 6. Detailed kinetic analysis of BLIP protein interacting with the TEM1 mutant protein TEM1 K234A. Shown is a set of six sensorgrams from vertical channel 4 (—, 600 nM; —, 300 nM; —, 150 nM; —, 75 nM; —, 37.5 nM; —, 18.8 nM) obtained in a single injection cycle using the ProteOn multiple protein interaction kit.

ProteOn Protein-Small Molecule Kit

The ProteOn protein-small molecule kit demonstrates the capability of the ProteOn XPR36 system to accurately measure and resolve the kinetics of a small molecule interaction with an immobilized protein.

The ProteOn protein-small molecule kit contains the enzyme inhibitor pair CA II and CBS (Figures 7 and 8). The affinity and kinetics of the interaction between CA II and CBS have been well characterized by SPR technology (Figure 9). The kit also supplies a reagent set designed for assessing the capability of the ProteOn XPR36 system to measure small molecule interactions and for establishing a benchmark for system performance.

The ProteOn protein-small molecule kit includes:

- CA II/CBS pair, with sufficient material to immobilize up to 5 channels with CA II and to analyze a complete concentration series of CBS
- GLC sensor chip
- Amine coupling kit
- Acetate buffer, 10 mM, pH 5.0
- Instruction manual

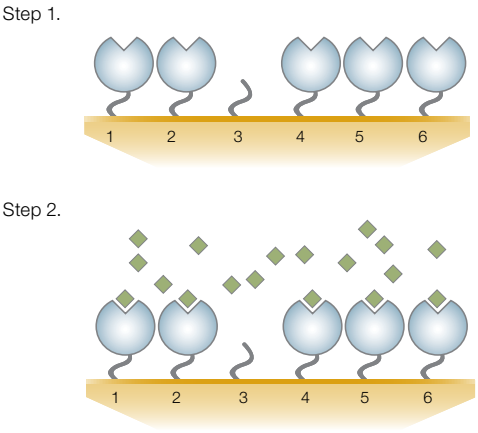

Fig. 7. Workflow of the ProteOn protein-small molecule kit. Step 1, immobilize CA II on the chip surface. Step 2, inject CBS analyte (MW 201), and monitor interaction with CA II (31 kD). (Channel 3 is a reference channel.)

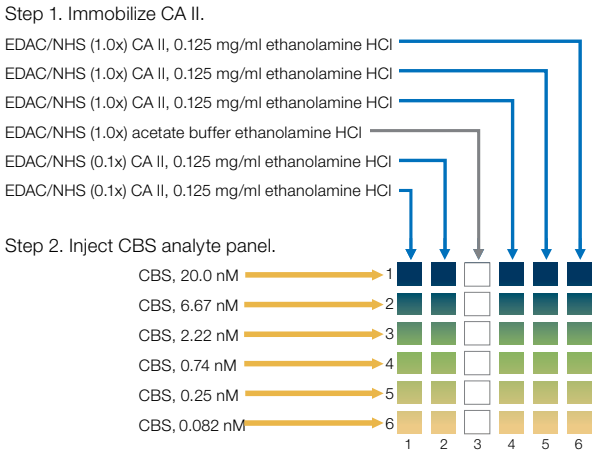

Fig. 8. ProteOn protein-small molecule kit representative protocol. Acetate buffer was at 10 mM, pH 5.0.

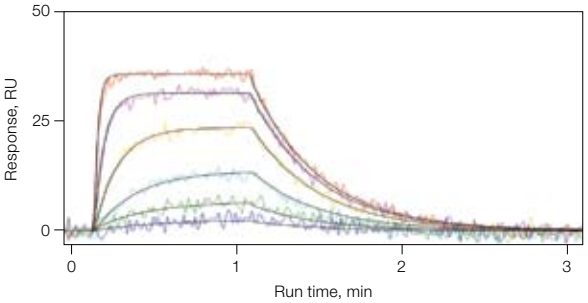

Fig. 9. Detailed kinetic analysis of the CA II/CBS interaction. Results obtained using the ProteOn protein-small molecule kit (—, 20 nM; —, 6.07 nM; —, 2.22 nM; —, 0.74 nM; —, 0.25 nM; —, 0.082 nM).

Ordering Information

Catalog #	Description
ProteOn Protocol Development Kits	
176-1010	**ProteOn One-shot Kinetics Kit**, includes IL-2/IL-2 antibody pair, GLC sensor chip, amine coupling kit, 50 ml acetate buffer, pH 4.5, instructions
176-1020	**ProteOn Multiple Protein Interaction Kit**, includes TEM/BLIP protein set, GLC sensor chip, amine coupling kit, 50 ml acetate buffer, pH 4.0, instructions
176-1030	**ProteOn Protein-Small Molecule Kit**, includes carbonic anhydrase II/CBS pair, GLM sensor chip, amine coupling kit, 50 ml acetate buffer, pH 5.0, instructions

To obtain bulletins 3172, 5358, and 5368, contact your local Bio-Rad sales representative or download them from **www.bio-rad.com**.

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