

STANDARD OPERATING PROCEDURE FOR THE COMMON FUND: PROTEIN CAPTURE REAGENTS PROGRAM (Surface Plasmon Resonance)

1. PURPOSE

This procedure is to be used to determine the binding affinity of an antibody to its target (antigen) by incorporating Surface Plasmon Resonance (SPR) technology on ProteOn™ XPR36 instrument.

2. SCOPE

This document describes the immobilization preparation (secondary (2°) antibody), sample preparation (primary (1°) antibody and antigen) and SPR procedure using ProteOn™ XPR36 instrument. The ProteOn™ XPR36 instrument is slightly different than the BIAcore T100 instrument in that the ProteOn™ XPR36 has a 6 x 6 chip which allows screening of five antibodies with one control (buffer) in real-time while providing an extra control (antigen specificity). The BIAcore T100 allows screening of one antibody with one control (buffer) in real-time.

3. RESPONSIBILITY

It is the responsibility of person(s) performing this procedure to be familiar with lab safety procedures and ProteOn™ Manager software. The interpretation of results must be done by a person trained in the procedure and familiar with such interpretation.

4. EQUIPMENT

- ProteOn™ XPR36 Protein Interaction Array System; Bio-Rad, Cat. #176-0100
- Vacuum Filtration Setup

5. MATERIAL

- ProteOn™ GLM Sensor Chip; Bio-Rad, Cat. #176-5012
- ProteOn™ MNT Maintenance Chip; Bio-Rad, Cat. #176-5100
- ProteOn™ Immobilization Buffer Kit; Bio-Rad, Cat. #176-2110
- ProteOn™ Regeneration and Conditioning Kit, Bio-Rad, Cat. #176-2210
- ProteOn™ Amine Coupling Kit; Bio-Rad, Cat. #176-2410

- ProteOn™ Chip Normalization Solution; Bio-Rad, Cat. #176-2810
- 1cc Syringe, Sterile, Disposable; VWR, Cat. #BD309659
- Acrodisc Syringe Filters, Supor w/PF* 25 mm, Diameter, 0.8/0.2 µm Pore Size, Sterile; PALL Corporation, Cat. #4187
- Nalgene® Rapid- Flow™ Sterilization Filter Units, Cellulose Nitrate Membrane; VWR, Cat. #28199-155
- 10X Phosphate buffered Saline (PBS); Fisher Scientific, Cat. #BP399-1 (diluted to 1X with deionized water; filtered)
- Tween-20; ACROS ORGANICS, Cat. #233362500
- Bovine Serum Albumin (BSA); Sigma, Cat. #A7030
- Deionized Water
- Assay Buffer (1X PBS, 0.05% Tween 20, 0.1% BSA and bring up to 1 L with deionized water; filtered)
- 1.5 mL micro-centrifuge tubes
- 96-Well Assay Blocks, Polypropylene, Corning®, 2 mL, V-bottom, non-sterile; VWR, Cat. #29445-166

6. REAGENTS

6.1. For mouse monoclonal antibody evaluation

- Antigen corresponding to the antibody.
- Purified monoclonal antibody corresponding to the Antigen
- Rabbit anti-mouse antibody (RAM); Jackson ImmunoResearch # 315-005-046

6.2. For human Fab binder evaluation

- Antigen corresponding to the antibody.
- Purified human Fab antibody corresponding to the Antigen
- Goat anti-human antibody (GAH); Jackson ImmunoResearch # 109-005-006

7. PROCEDURE

7.1. QC Buffer Injections (perform before runs)

7.1.1. Remove ProteOn™ Hydrochloric Acid (HCl), ProteOn™ Sodium Hydroxide (NaOH), ProteOn™ Sodium Dodecyl Sulfate (SDS) and new GLM chip from 4°C and equilibrate to Room Temperature (RT). Prepare 1L 1X PBS and filter using Nalgene® Rapid- Flow™ Sterilization Filter Units.

- 7.1.2. Insert RT equilibrated GLM chip into ProteOn™ XPR36 instrument.
- 7.1.3. Perform Air Initialization on RT equilibrated GLM chip according to the ProteOn™ Manager Manual. Ensure leads are in deionized water bottle. After Air Initialization, the Initialization Status will be green and Instrument State located below screen reads Ready.
- 7.1.4. Once Air Initialization has completed, design or copy a Conditioning protocol according to the ProteOn™ Manager Manual. The conditioning of a new chip is optional, but highly recommended. A typical Conditioning protocol includes sequentially SDS injections horizontally at 30 $\mu\text{L}/\text{min}$ Flow rate x 60 s Contact time = 30 μL Volume; NaOH injections horizontally at 30 $\mu\text{L}/\text{min}$ Flow rate x 60 s Contact time = 30 μL Volume; HCl injections horizontally at 30 $\mu\text{L}/\text{min}$ Flow rate x 60 s Contact time = 30 μL Volume; repeat Conditioning injections vertically.
- Note:** This is not a complete protocol. See ProteOn™ Manager Manual for complete details.
- 7.1.5. Once chip conditioning has completed, design or copy a QC Buffer Injection (1X PBS, filtered) protocol according to the ProteOn™ Manager Manual. A typical QC Buffer Injections protocol includes sequentially Buffer injections horizontally (4x) at 100 $\mu\text{L}/\text{min}$ Flow rate x 60 s Contact time = 100 μL Volume ; 0 s Dissociation time; repeat Buffer injections vertically (4x).
- Note:** This is not a complete protocol. See ProteOn™ Manager Manual for complete details.
- 7.1.6. Place buffer and water bottles appropriately in ProteOn™ XPR36 instrument. Ensure leads (A or B) are placed appropriately in buffer and water bottles.
- 7.1.7. Add buffer to assay block wells according to lay out and place assay block in position 1(near white box) of ProteOn™ XPR36 instrument.
- 7.1.8. Click Run and select your QC Buffer Injection protocol under Select Protocol/Experiment drop box. Click Start and follow prompts below screen. You will be prompt several times to click Start.

7.1.9. After QC Buffer injections, sensorgrams should appear on screen.

If

not, open your QC Buffer Injection protocol and sensorgrams should appear. Perform data analysis according to ProteOn™ Manager Manual and view sensorgrams to confirm good injections. If good injections are not achieved, repeat another run of QC Buffer injections.

7.2. Immobilization of 2° Antibody on New GLM Chip

7.2.1. Once new chip has been Air Initialized, Conditioned (optional) and QC Buffer Injections performed (see section 7.1), remove ProteOn™ Immobilization Buffer Kit and ProteOn™ ethanolamine-HCl from 4°C and equilibrate to RT. Remove ProteOn™ Amine Coupling Kit from -20°C and follow manufacturer's protocol for dissolving, aliquoting and storing EDAC and sulfo-NHS stock solutions.

7.2.2. Prepare 2° antibody for immobilization onto GLM chip. If screening human Fab antibodies, prepare a 2 mL dilution of GAH antibody into ProteOn™ Acetate Buffer pH 4.5 to give a final concentration of 25 µg/mL. If screening mouse monoclonal antibodies, prepare a 2 mL dilution of RAM antibody into ProteOn™ Acetate Buffer pH 5.0 to give a final concentration of 25 µg/mL.

Note: Concentration of the 2° antibody may need to be adjusted depending on the individual antibody performance.

7.2.3. Prepare fresh Activation Solution by mixing 100 µL of the 10X EDAC stock solution, 100 µL of the 10 X sulfo-NHS stock solution and 1800 µL of deionized water. Discard any unused EDAC and sulfo-NHS stock solutions.

7.2.4. Design or copy an Immobilization protocol according to ProteOn Manager Manual. A typical Immobilization protocol includes sequentially EDC/NHS injections horizontally at 50 µL/min Flow rate x 300 s Contact time = 250 µL Volume; Ligand (RAM or GAH antibodies) injections horizontally at 30 µL/min Flow rate x 300 s Contact time = 150 µL Volume; Ethanolamine-HCl injections horizontally at 30 µL/min Flow rate x 300 s Contact time = 150 µL Volume.

Note: This is not a complete protocol. See ProteOn™ Manager Manual for complete details.

- 7.2.5. Ensure leads are placed correctly into appropriate buffer and water bottles on ProteOn™ XPR36 instrument.
- 7.2.6. Click Run and select your Immobilization protocol under Select Protocol/Experiment drop box. Click Start and follow prompts below screen. You will be prompt several times to click Start.
- 7.2.7. Once Immobilization protocol has completed, analyze data and confirm ≥ 5000 Response Units (RU) is achieved for each channel. If ≥ 5000 RU is not achieved for each channel, another chip may need to be immobilized.

7.3. Assay Run

- 7.3.1. After a successful Ligand Immobilization (2° antibody) on chip (≥ 5000 RU achieved/channel), an assay can be run. Remove ProteOn™ H3PO4 from 4°C and equilibrate to RT. Prepare Assay Buffer (filtered), primary antibodies and antigens corresponding to the antibodies. Use Nalgene® Rapid- Flow™ Sterilization Filter Units to filter Assay Buffer; usually 1 - 2 L is needed per assay run.
- 7.3.2. For each 1° antibody to be evaluated prepare a 500 μ L dilution in filtered Assay Buffer to give a final concentration of 20 μ g/mL. Filter each antibody dilution with 1cc syringe and pre-wetted Acrodisc syringe filter. Use filtered Assay Buffer to pre-wet Acrodisc syringe filter.
Note: Concentration of the 1° antibody may need to be adjusted depending on the individual antibody performance.
- 7.3.3. For each antigen prepare a 500 μ L dilution in filtered Assay Buffer to give a final concentration of 1 μ M. Antigen filtration is not required.
Note: Concentration of the antigen may need to be adjusted depending on the individual antigen performance.

- 7.3.4. The Instrument State located below screen will read Stand-by and will need to be resumed. Resume ProteOn™ XPR36 instrument according to ProteOn™ Manager Manual.
- 7.3.5. Perform Glycerol Initialization on immobilized chip according to ProteOn™ Manager Manual. Glycerol Initialization takes about 5 -7 min.
- 7.3.5.1. If Glycerol Initialization passes, the Initialization Status will be green and Instrument State located below screen will read Ready. If Glycerol Initialization does not pass, Initialization Status will be red and Instrument State located below screen will read Not Ready.
- 7.3.5.2. For failed Glycerol Initializations, eject chip and gently rinse chip with deionized water. Gently wipe dry with KimWipes and ensure chip is completely dry and nothing is on it. Insert chip back into ProteOn™ XPR36 instrument and restart Glycerol Initialization.
- 7.3.6. Once chip has passed Glycerol Initialization, an assay can be run. Design or copy an Assay protocol according to ProteOn™ Manager Manual. A typical Assay protocol includes sequentially Ligand (1° antibody) injections vertically at 30 µL/min Flow rate x 300 s Contact time = 150 µL Volume; Analyte (antigen) injections horizontally at 30 µL/min Flow rate x 120 s Contact time = 60 µL Volume and 120 s Dissociation time; H3PO4 injections horizontally (2x) at 100 µL/min Flow rate x 18 s Contact time = 30 µL Volume; H3PO4 injections vertically at 100 µL/min Flow rate x 18 s Contact time = 30 µL Volume; Buffer injections horizontally at 100 µL/min Flow rate x 60 s Contact time = 100 µL Volume and 0 s Dissociation time; Buffer injections vertically at 100 µL/min Flow rate x 60 s Contact time = 100 µL Volume and 0 s Dissociation time.
- Note:** This is not a complete protocol. See ProteOn™ Manager Manual for complete details.
- 7.3.7. Remove and discard used assay block from ProteOn™ XPR36 instrument. Remove QC Buffer from ProteOn™ XPR36 instrument and replace with Assay Buffer (filtered). Ensure leads are placed

correctly into appropriate buffer and water bottles on ProteOn™ XPR36 instrument.

7.3.8. Add samples to new assay block wells according to lay out and place assay block in position 1(near white box) of ProteOn™ XPR36 instrument. Any sample that has a zero for required volume is excluded from run and no action is required. If Assay requires two assay blocks, place second assay block behind first assay block.

7.3.9. Click Run and select your Assay protocol under Select Protocol/Experiment drop box. Click Start and follow prompts below screen. You will be prompt several times to click Start.

7.3.10. After assay run, perform data analysis according to ProteOn™ Manager Manual.

7.4. Post-Experimental Clean-Up

7.4.1. When assay run has completed and no more assays will be run on chip, a Post-Experimental Clean-Up is required.

7.4.2. Eject chip and place back in package foil and box and store at 4°C for later use. Remove and discard assay block(s) and Assay Buffer from ProteOn™ XPR36 Instrument. Wipe Assay Buffer leads with KimWipes and place in deionized water bottle. Ensure all leads are in deionized water bottle.

7.4.3. Insert MNT chip into ProteOn™ XPR36 instrument. Follow Post-Experiment Clean-Up protocol according to ProteOn™ Manager Manual. Once Post-Experiment Clean-Up has completed, the Instrument State located below screen will read Inactive. Do not turn off ProteOn™ XPR36 instrument.

7.5. Weekly Clean-Up

7.5.1. At the end of the week, perform Weekly Clean-Up with MNT chip inserted in ProteOn™ XPR36 instrument.

7.5.2. Perform Weekly Clean-Up according to ProteOn™ Manager Manual. Once Weekly Clean-Up has completed, the Instrument

State located below screen will read Inactive. Do not turn off ProteOn™ XPR36 instrument.

8. **REFERENCED DOCUMENTS**
- 8.1.1. Operation Manual, ProteOn™ XPR36 Instrument
 - 8.1.2. Operation Manual, ProteOn™ Manager Software
 - 8.1.3. Instruction for use, ProteOn™ Amine Coupling Kit