ProteinChip® Array
Quality Control of Antibodies

ProteinChip(R) arrays and the PBSII system can be used for an "SDS-PAGE" like analysis to:

- check purity
- analyze for presence or absence of a particular molecule
- test the ability of proteins to be detected (i.e. ionized)

Quality Control:

1. Add 1µl of protein solution to each spot on a NP20 or H4 (preferred for antibody QC) array. The ideal protein concentration is 200-2000nM (10 fold less for peptides)
2. Dry protein solution onto chip surface. If protein or antibody is pure and there are no contaminating substances in the buffer such as salts or detergents proceed to step 4.
3. If buffer contains detergents or salts, add 5µl of distilled water to the spot and draw liquid in and out 5-10 times.
4. Air dry the chip array and add 1 ul EAM. Analyse using the SELDI ProteinChip reader.

ProteinChip® Arrays H4 & H50 (Reverse Phase)

Direct Spotting:

1. Draw an outline for each spot using a hydrophobic pen (only for H4 chips).
2. For H4 chips pretreat the spots with 2 µl of 50% acetonitrile for 1-2 min, remove and add 2 µl of PBS for 1-2 min.

   For H50 chips pretreat the spots with 2 µl of 50% acetonitrile for 5 min, remove and repeat treatment once.

3. Add 2 µl of Binding buffer to each spot incubate for 5 min
   a. PBS + 0.5M NaCl (least stringent)
   b. PBS (mild stringency)
   c. 10-50% ACN/PBS

   *Add 0.1% TFA to the binding buffer when using H50 chips.

4. Remove BB and replace with 1-7µl of sample (diluted in BB). Do not allow the spots to air dry during sample exchange.
5. Incubate in a humidity chamber for 30 min to 1.5 hrs at RT.
6. Wash each spot with 5µl of binding buffer three times
7. Do a final 'quick wash' with 5 mM Tris pH 8 or 10 mM Hepes pH 7.4, let spots dry.
8. Add 1µl of saturated EAM solution to each spot.
9. Analyse the chip using SELDI PBSII.

Bioprocessor:

1. Draw an outline for each spot using a hydrophobic pen (only for H4 chips).
2. Pretreat the spots with 2 µl of 50% acetonitrile for 1-2 min, remove and add 2 µl of PBS for 1-2 min.

   For H50 chips pretreat the spots with 2 µl of 50% acetonitrile for 5 min, remove and repeat treatment once.

3. Put chips into bioprocessor and add 50 µl of BB to each well and incubate for 5 min shaking at RT.

4. Remove BB and add 50 – 350 µl of sample (diluted in BB). Do not allow the spots to air dry during sample exchange
5. Incubate shaking for 30 min to 1.5 hrs at RT.
6. Wash each spot with 200 µl of binding buffer for 5 min while shaking, repeat times.
7. Do a quick wash with low molarity buffer 5 mM Tris pH 8 or 1-10 mM Hepes pH 7.4, dry spots.
8. Add 1ul of saturated EAM solution to each spot.
9. Analyze the chip using SELDI PBSII.

Recommended Buffers

0-60% acetonitrile ± 0.1% TFA. Isoproponal or methanol can also be used. Increasing the solvent concentration will increase the selectivity of the surface. Salts will increase hydrophobic interactions and therefore can also be included in the binding buffer (50-1000mM).

Additional Notes:

A humidity chamber can be assembled by half filling an empty tip-box with water and placing damp tissue paper in the lid. The Proteinchip® array can be placed on the tip-support in this chamber to prevent evaporation from the spots

ProteinChip® Array SAX-2
(Strong Anion Exchanger)

The SAX-2 chip contains quarternary ammonium groups (strong cationic moieties) on the surface. The surface is prepared simply by equilibrating the spots in the binding buffer.

1. Draw an outline for each spot using hydrophobic pen.
2. Load 5µl of binding buffer to each spot and incubate in a humidity chamber at room temperature for 5 min. Do not allow spots to air dry.
3. Remove excess buffer from spots without touching the active surface.
4. Load 1-7µl of sample per spot. Sample should be diluted in the binding buffer. Typical concentrations vary depending on the complexity of the sample. A non-ionic detergent can be included in the binding and washing buffers (e.g. 0.1% OGP or Triton X-100) to reduce non-specific binding. Varying the pH and ionic strength of the binding and/or washing buffer can also modify ionic binding.
    a. 50mM Tris pH 9 (least stringent)
    b. 50mM NaAcetate pH4.5 (most stringent)
5. Incubate in a humidity chamber for 30 min to 1.5 hrs at RT.
6. Wash each spot with 5µl of BB three times then a final wash with 5 mM Tris pH 8 or 1-10 mM HEPES pH 7.4.
7. Wipe dry around spots. Add 1ul of saturated EAM solution to each spot.
8. Analyze chip using SELDI PBSII.

Recommended Buffers

20 to 100mM sodium or ammonium acetate, Tris HCl or 50mM Tris base (for pH > 9) buffers containing a non-ionic detergent (e.g. 0.1% Triton X-100). Decreasing the pH of the buffer or increasing the salt content will increase the binding specificity (selecting for strongly anionic proteins).

ProteinChip® array WCX-2
(Weak Cation Exchanger)

The WCX-2 chip contains carboxylate groups (weak anionic moieties) on the surface and is shipped in the salt form with sodium as the counter-ion. In order to minimize the sodium adduct peaks in the mass spectra, it is recommended that the chip be pretreated before loading the sample.

1. Pretreat chip by washing with 10ml of 10mM hydrochloric acid for 10 minutes shaking. Rinse with 10ml of HPLC grade water for 10 min shaking. Wipe dry around spots.
2. Draw an outline for each spot using hydrophobic pen.
3. Load 5µl of Binding Buffer to each spot and incubate in a humidity chamber at room temperature for 5 min. Do not allow buffer to air dry.
4. Remove excess buffer from spots without touching the active surface.
5. Load 1-7µl of sample per spot (sample should be diluted in the binding buffer) and incubate the chip in a humidity chamber for 30 min to 1.5hrs at RT.
    a. 50mM Tris pH 9 (most stringent pH)
    b. 50mM NaAcetate pH 4.5 (least stringent pH)
6. Wash each spot with 5µl of BB three times then a final wash with 5 mM Tris pH 8 or 1-10 mM HEPES pH 7.4.
7. Wipe dry around spots. Add 1ul of saturated EAM solution to each
8. Analyze chip using SELDI PBSII.

Recommended Buffers

20 to 100 mM ammonium acetate and phosphate buffers containing low concentration of a non-ionic detergent (e.g. 0.1% Triton X-100). Increasing the pH of the buffer or the salt content will increase the binding specificity (selecting for cationic proteins).

ProteinChip® array IMAC-3
(Immobilized Metal Affinity Capture)

The IMAC-3 chip contains nitrilotriacetic acid (NTA) groups on the surface. It is manufactured in the metal-free form and must be loaded with metal, i.e. Cu, prior to use.

* Copper is somewhat corrosive to the chip surface and should not be left on the spots longer than the recommended time.

1. Draw an outline for each spot using hydrophobic pen.
2. Load 10µl of 100mM copper sulfate to each spot and incubate in a humidity chamber for 15 min. Do not allow solution to air dry. Repeat loading.
3. Rinse chip under running deionized water for about 10 seconds to remove excess copper.
4. Rinse spots with an excess of 50 mM sodium acetate, pH 4.0.
5. Rinse chip again under running deionized water for about 10 seconds .
6. Add 5µl of 0.5M NaCl in PBS (or other binding buffer containing at least 0.5M NaCl) to each spot and incubate for 5 minutes. Do not allow buffer to air dry. Wipe dry around spots and remove excess buffer without touching the active surface.
7. Load 1-7 µl of sample per spot (sample should be diluted in BB). Incubate chip in a humidity chamber for 30 minutes to 1.5 hrs at RT.
8. Wash each spot with 5µL of binding buffer three times, followed by a quick wash with water (5µl) or 1-10 mM HEPES pH 7.4.
9. Add 1 µl of saturated EAM solution to each spot.
10. Analyze chip using SELDI PBSII.

Recommended Buffers

A binding buffer containing sodium chloride (at least 0.5M) and detergent (e.g. 0.1% Triton X-100) is recommended to minimize non-specific ionic and hydrophobic interactions, respectively. EDTA and DTT should be avoided in the sample buffer.

General Sample Preparation

Detergents:	avoid ionic detergents such as SDS or its derivatives Triton X-100 and NP40 can be used, it is best to have no more than 0.1% detergent in the final diluted sample you apply to the array
Protein Concentrations:	It is generally a good idea to keep the lysates or other protein samples fairly concentrated, so that you can dilute them into the appropriate buffer for each chip surface. I recommend a concentration of at least 2 mg/ml, which allows a 10 fold dilution into appropriate buffers. Lower concentrations can be used and diluted less, but it may limit your choice of array surfaces.
Chemicals to avoid:	PEG is difficult to wash off and will exhibit a broad peak High concentrations of glycerol will prevent the protein from binding, you can dilute the sample to about 5% glycerol EDTA will interfere with binding to the IMAC array

Lipids will also interfere, so include a high speed centrifugation in your sample prep if your sample might contain high concentrations of lipids.

General comments: You can bind under one condition and wash the chip with higher stringency, but you will get improved specificity if the binding and washing conditions are the same. It is easier to prevent binding than to completely remove bound components.

Retentate Mapping of Crude Biological Samples Using a Bioprocessor

Standard Protocol

1. Place chip in bioprocessor (*see protocol on handling the bioprocessor*) and add 50-100µl of binding buffer to each well. Incubate for 5 min at room temperature on vortex (shaker setting).
2. Remove buffer from well and immediately add sample which has been diluted into binding buffer (recommended vol 50ul to 350 ul of diluted sample). Incubate on a shaker for 30 min to 1.5 hrs at RT. Total volume for 96 well bioprocessor is 350 µl.
3. Remove sample from well and wash each well with 3 x 200µl of binding buffer, 5 min each wash shaking. Do a final wash with a low molarity buffer such as 10 mM Hepes pH 7.4 for 30sec while shaking at RT.
4. Air dry the chip and add 1 µl of saturated EAM.

Washing buffers for specific surfaces

The above protocol can be used to adapt any retentate mapping experiment to the bioprocessor. Initial experiments on a sample should involve the analysis of a range of binding/washing conditions.