



ProteinChip® Protocol for Antibody Capture

--Direct Covalent Coupling of Antibody to PS10 or PS20 activated ProteinChips®

Important Notes:

Antibodies should be of high quality and work in either ELISA, FACS, or IP conditions.

Antibodies should *NOT* be in Tris based buffers, should *NOT* have 'protein stabilizers' such as BSA or gelatin, nor preservatives such as sodium azide.

If antibodies have Tris, BSA, or gelatin, please go to Indirect Coupling of Antibodies through Protein G. If antibodies have sodium azide you may proceed but you may have to allow the azide to degrade or to dilute the antibody.

You may use either monoclonal or polyclonal antibodies that have been column purified.

All antibodies or protein must be QC before covalent attachment to the chip. This involves checking the integrity of the antibody by MS not by gel. You should spot 0.5 to 1ug of antibody on a H4 (for proteins it can be NP2 chip) chip, allow the chip to dry and add SPA. Look for peaks at 150kD (single charge) and 75kD (double charge). There should be little to no peaks at 50kD (heavy chain) or 22kD (light chain). If there are your antibody is BROKEN down. The laser CANNOT disrupt an antibody into the heavy and light chain. Your reagent is degraded.

Procedure:

PS10: General information

- a. Pre-activated surface has carbonyl diimidazole (CDI) activated amine surface
- b. Covalently binds proteins, peptides through an amine group
- c. High binding capacity
- d. Binding of samples and washes must be more stringent than PS20

Buffers

- a. Typically use higher detergent concentrations
- b. Low stringency: PBS + 0.5% Triton X-100
- c. High stringency: 50mM Tris pH 8 + 1% Triton X-100
- d. Generally stay away from high salt concentrations in buffers when using PS10 chips.

Protocol: PS10

- a. Briefly wet each spot with 2ul of 50% acetonitrile/water (all water should be HPLC grade or better) for 1 min
- b. Take off with the edge of a kimwipe. Add 2ul PBS and incubate at RT in a humidity box for 1 min
- c. Take off with the edge of a kimwipe.
- d. Add 2 ul of a carbonate buffer pH 9.0 to each spot and then add 1-2 ul of 1- 3 ug of antibody or protein (if the antibody solution is dilute repeated applications can be done or consider concentrating the antibody).
- e. Place in a humidity box for 1-2hrs at RT
- f. Take off the antibody with the edge of a kimwipe.



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- g. In a 15 ml conical tube add 7 ml of 1 M ethanolamine/PBS pH 8 or 1 M Tris pH 8 and slide chip into the tube. Incubate on a shaking platform for at least 30 min
- h. Dump out solution being careful not to disturb the chip in the tube.
- i. Wash chip two times with PBS + 0.5% TX-100 at RT, 5 min while shaking

Protocol: PS10 Sample Addition – Direct Sample Addition

- a. Dry around the spot as much as possible.
- b. Add up to 7 ul of sample. (You should determine the concentration of your protein sample. The amt you will add is going to be empirical, if you have a purified protein, then you will use less. If you have a complex sample, you will use a higher concentration. Remember this all depends on the affinity of the antibody to the antigen). Usually you will dilute your sample into Binding Buffer (BB), see above for typical BB used with PS10 chips.
- c. Incubate in a humidity box at RT for 2 hrs or 4°C O/N. Sometimes it might be prudent to do the 2hr incubation at 4°C due to affinity and on-off rates of antibody:antigen.
- d. Carefully remove the sample with a pipet or with a kimwipe.
- e. Add 5ul of BB and pipet up and down at least 15 times
- f. Remove the BB, repeat wash two more times
- g. Add 5ul of PBS and pipet up and down at least 15 times
- h. Remove the PBS
- i. In a 15 ml conical tube add 7 ml of 10 mM HEPES pH 7.4, slide chip into tube
And rinse for 30 seconds
- j. Allow chip to dry, add EAM, read

Protocol: PS10 Sample Addition – Bioprocessor

- a. Dry around the spot as much as possible and slide the chip into the bioprocessor, note where the top of the chip is and the bioprocessor
- b. The amount you add again will be empirical and will be dependent heavily on the affinity of the antibody to the antigen. Start with smaller volumes if possible with high concentrations. Keep the volume at less than 125ul (for the 96 well bioprocessor). Dilution should be with the above recommended BB
- c. Incubate at RT while shaking for 1-2 hours. Once again, you might have to vary the temperature or the time depending on the affinity of the antibody to the antigen.

Wash twice with BB at RT for 3 min while shaking. This will vary depending on the non-specificity seen. At first start with **short washes**, once you have seen the antigen then increase wash times and stringencies

- d. Wash once with PBS at RT for 3 min while shaking
- e. Wash once with 10mM HEPES pH 7.4 for 30 sec.
- f. Dump out, remove chip from bioprocessor, dry, add EAM and read



Procedure:

PS20 chips: General Information

- a. Pre-activated surface with an epoxide moiety
- b. Covalently binds proteins/peptides through amine group
- c. Low binding capacity
- d. Binding of samples and washes must be less stringent than PS10

Buffers

- a. Typically use lower detergent concentrations
- b. Low stringency: PBS + 0.1% Triton X-100
- c. High stringency: 50mM Tris pH 8 +150mM NaCl + 0.5% Triton X-100

Protocol: PS20

- a. Add 2 ul of a carbonate buffer pH 9.0 to each spot and then add 1-2 ul of 1- 3 ug of antibody or protein (if the antibody solution is dilute repeated applications can be done or consider concentrating the antibody).
- b. Place in a humidity box for 1-2hrs at RT
- c. Take off the antibody with the edge of a kimwipe.
- d. In a 15 ml conical tube add 7 ml of 1 M ethanolamine/PBS pH 8 or 1 M Tris pH 8 and slide chip into the tube. Incubate on a shaking platform for at least 30 min
- e. Dump out solution being careful not to disturb the chip in the tube.
- f. Wash chip two times with PBS + 0.1% TX-100 at RT, 5 min while shaking

Protocol: PS20 Sample Addition – Direct Sample Addition

- a. Dry around the spot as much as possible.
- b. Add up to 7 ul of sample. (You should determine the concentration of your protein sample. The amt you will add is going to be empirical, if you have a purified protein, then you will use less. If you have a complex sample, you will use a higher concentration. Remember this all depends on the affinity of the antibody to the antigen). Usually you will dilute your sample into Binding Buffer (BB), see above for typical BB used with PS20 chips.
- c. Incubate in a humidity box at RT for 2 hrs or 4°C O/N. Sometimes it might be prudent to do the 2hr incubation at 4°C due to affinity and on-off rates of antibody:antigen.
- d. Carefully remove the sample with a pipet or with a kimwipe.
- e. Add 5ul of BB and pipet up and down at least 15 times
- f. Remove the BB, repeat two more times
- g. Add 5ul of PBS and pipet up and down at least 15 times
- h. Remove PBS
- i. In a 15 ml conical tube add 7 ml of 10 mM Hepes pH 7.4, slide chip into tube
And rinse for 30 seconds
- j. Allow chip to dry, add EAM, read

Protocol: PS20 Sample Addition – Bioprocessor

- a. Dry around the spot as much as possible and slide the chip into the bioprocessor, note where the top of the chip is and the bioprocessor
- b. The amount you add again will be empirical and will be dependent heavily on the affinity of the antibody to the antigen. Start with smaller volumes if possible with high concentrations.



Keep the volume at less than 125ul (for the 96 well bioprocessor). Dilution should be with the above recommended BB.

- c. Incubate at RT while shaking for 1-2 hours. Once again, you might have to vary the temperature or the time depending on the affinity of the antibody to the antigen.
- d. Wash twice with BB at RT for 3 min while shaking. This will vary depending on the non-specificity seen. At first start with short washes, once you have seen the antigen then increase wash times and stringencies
- e. Wash once with PBS at RT for 3 min while shaking
- f. Wash once with 10mM Hepes pH 7.4 for 30 sec.
- g. Dump out, remove chip from bioprocessor, dry, add EAM and read

--Indirect Coupling of Antibody to PS10 or PS20 activated biochips

Protein A, Protein G or Protein A/G must be used when in-direct coupling of antibody to the chip will be done. Indirect coupling is normally used when the antibody is not clean (anti-sera, ascites, protein stabilizers present, Tris buffer).

The advantage in using protein A or G to couple the antibody to the chip is that you will not have to purify your antibody and your antibody will automatically be in the correct orientation on the chip.

The disadvantage is that there will be extra time spent on extra steps, lower binding capacity and less specificity due to non-specific interactions of protein A or G with proteins.

Protein A or G must follow the same requirements as stated above for antibodies. That is the protein should be solubilized in PBS (pH 7.4). No Tris containing buffers.

Protein G Coupling: PS10 or PS20 chip

- a. On to dry chip surface (PS20) spot 4ul of 0.5ug/ul Protein G, incubate overnight in a humidity chamber at 4°C.
- b. If using PS10 you must prepare the surface prior to coupling: Briefly wet each spot with 2ul of 50% acetonitrile/water (all water should be HPLC grade or better) for 1 min. Take off with the edge of a kimwipe. Add 2ul PBS and incubate at RT in a humidity box for 1 min. Take off with the edge of a kimwipe. Add protein G as above and incubate overnight in a humidity chamber at 4°C
- c. AM: if the spot is slightly dry add 4ul of ddH₂O and allow to incubate at RT in a humidity chamber, blot off
- d. In a 15 ml conical tube add 7 ml of 1M ethanolamine/PBS pH 8 for 30 min, rocking at RT
- e. Dump out, add 7ml 0.1% TX-100/PBS, rock at RT for 5 min, repeat
- f. Dry chip on the front and back being careful of the spots
- g. Add 1-3ug of antibody to the chip
- h. Incubate at RT for 1-2hrs, in a humidity chamber
- i. Blot off
- j. In bulk washes add 7ml of 0.1% TX-100/PBS, rock at RT for 5 min, repeat (while shaking)



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- k. In bulk wash of 7ml wash once with PBS, rock at RT for 5 min while shaking
- l. In bulk wash of 7ml wash once with 10 mM HEPES pH 7.5 for 30 sec while shaking
- m. Remove the chip from the conical tube, dry the spots and add the sample as above