SuperChip AdvaNCe – Protocol for Antibody Microarray

Printing:
Dilute protein in appropriate print buffer. Optimize buffer to ensure that protein properties are not adversely affected. Use PBS with 5 - 15% glycerol when printing antibodies. Other suitable array buffers that promote stability of proteins may also be used.

- Do NOT pre-wet slides, as this may cause arrayed spots to spread out onto the surface. Print protein onto slides. Printing is done at room temperature. Relative humidity should be adjusted to keep source plate from drying out, and pins from drying out while printing. Humidity of 65% has been evaluated in antibody arrays using SuperChip AdvaNCe substrates. Please choose your humidity settings based on arrayer needs, keeping in mind that too high humidity may lead to water condensation on the slide surface.

- After printing, slides are stored overnight in desiccant chamber at room temperature to optimize protein binding. If the protein is sensitive to temperatures, it should be stored appropriately. Slides should be stored in the dark, to protect the fluorophor from bleaching.

Blocking:

- Slides should be blocked for at least 1 hour in TBST (TBS with 0.1% Tween-20) buffer with some agitation. The concentration of TBST can be increased slightly, if necessary.

- TBST buffer should be drained off, and fresh TBST buffer added for 3 - 5 minutes. Experimentation should be done to determine optimal blocking buffer and concentration when performing fluorescent assays.

Blocking buffers containing BSA, milk solids, etc. should be tested prior to use. Some buffers containing protein add to the fluorescent background. Casein-containing blocking buffers may increase potential for ‘speckles’ or precipitates on the slides due to low solubility attributes of casein.

- Rinse slides in diH2O for 2 minutes.

- Do not wait too long before proceeding to next step.

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Target incubation:
Dilute labeled target to appropriate concentration in appropriate buffer. PBS with 0.05% Tween-20 is one choice. Choose the buffer that will maintain protein properties. Others have also successfully used PBS (pH 7.2-7.5) containing 0.05% Tween 20, 0.1% BSA, and included other additives such as protease inhibitors. Prepare enough to incubate the slides.

For ELISA-type arrays, samples may be diluted in same buffer. For optimization, different dilutions should be tested.

- Use some type of incubation chamber - LifterSlips will not work due to the hydrophobic nature of the nitrocellulose surface.
- Add the target (in incubation buffer) to the slides using a pipette. Ensure entire array area is covered (buffer will not necessarily spread on its own because surface is fairly hydrophobic).
- Seal the incubation chamber(s) to prevent solution from drying out
- Incubate at room temperature in the dark for 2 hours – overnight, depending on the type of assay being performed. For simple experiments similar to Western Blots (probing antibody with sample) a 2-hour incubation is usually sufficient. If possible incubate with very light agitation.

Post-processing:
Do not dry slides between wash steps

- Remove chamber covers and dump target solution from slides
- Put slides into PBST (PBS with 0.1% Tween-20) with moderate agitation for 5 minutes (repeat twice, with fresh solution each time).
- Wash slides in PBS for 5 minutes.
- Wash slides in diH2O for 2 minutes.
- Dry slides carefully with clean air, or by centrifugation. Damage to the surface will cause increases in background signal.
- Store slides in light protective box or folder until scanning.

Imaging:

Due to the nature of nitrocellulose film slides, PMT settings (voltage and gain) will need to be lower than traditional glass slides. For optimal results, these settings should be determined based on your individual assays and imaging equipment.