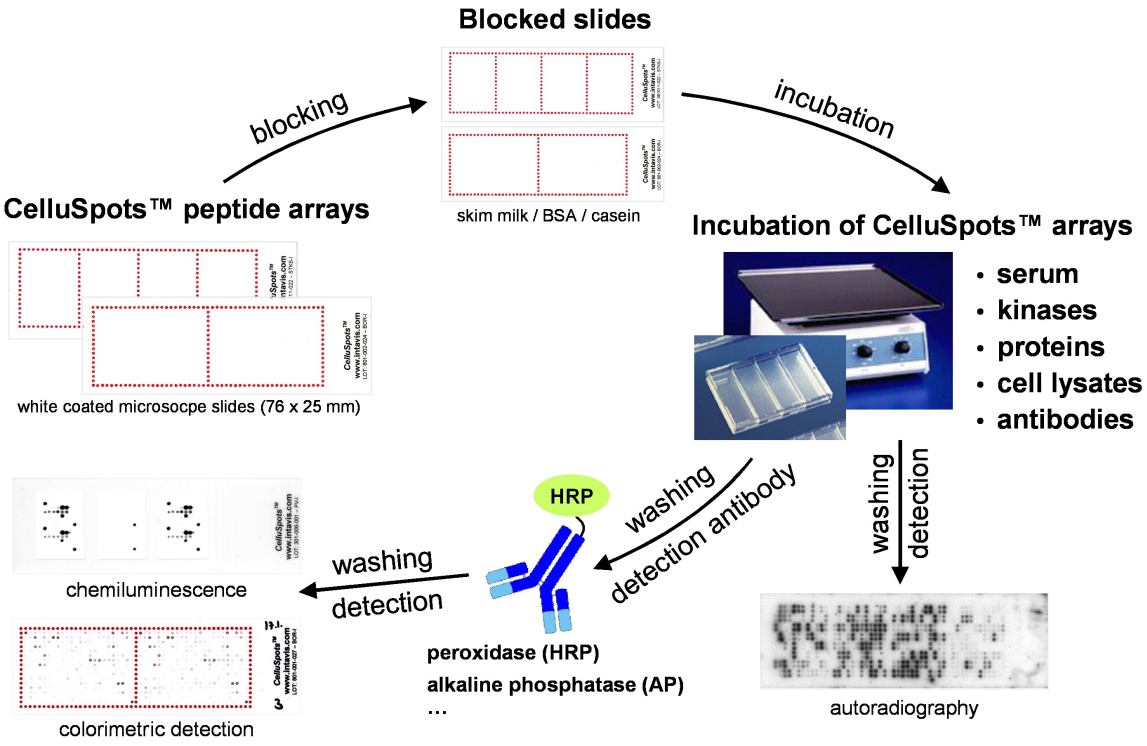
CelluSpots™ Peptide Arrays

1 Handling and storage

The CelluSpots™ peptide arrays should be kept in a dry and dark place at 4°C. Under these conditions the arrays are stable for at least 3 months. The surface of CelluSpots™ arrays is stable under incubation conditions but sensitive to mechanic stress and should not be touched or wiped.

CelluSpots™ peptide incubation / detection – schematic procedure:



2 Protocols

2.1 Blocking

To prevent non-specific binding, the arrays should be blocked by immersing the slides in blocking solution for 1-4 h at room temperature on an orbital shaker. Alternatively, block overnight at 4°C.

2.2 Primary antibody or protein incubation

Dilute the primary antibody, serum or protein in blocking solution. The dilution factor should be determined empirically for each antibody or protein (a typical dilution of serum might be in the range of 1:10 to 1:1000). Incubate the peptide array with diluted antibody or protein solution for 3-8 h in an incubation chamber (e.g. "4 Well Rectangular Dishes", NUNC can be used to incubate 4 arrays in parallel) on an orbital shaker or under a cover slip in a humidity chamber at room temperature. Alternatively the slides can be incubated over night at 4°C. Remove the cover slip – if incubation was done under a cover slip – carefully by rinsing with blocking solution. Wash the slides as described under ***2.3***.

NOTE: Due to better signal intensities it is strongly recommended to perform incubation and washing of CelluSpots™ arrays in incubations chambers on an orbital shaker. For NUNC plates ("4 Well Rectangular Dishes") approx. 2-3 ml are needed to cover the slides during washing and incubation steps.

2.3 Washing

The optimal washing times and Tween-20 concentrations may vary, depending on the antibodies or proteins used for the binding assay. For most applications TBS-T or PBS-T buffers with 0.05% Tween-20 are sufficient. Wash the slide 30 sec with TBS-T or PBS-T washing buffer, followed by three washing steps in TBS-T or PBS-T, 5 min each.

2.4 Secondary antibody incubation

Depending on your detection system secondary antibodies can be labeled with AP, HRP, biotin, fluorescent dyes or other reporter groups. To find the optimal concentration, different dilutions of the secondary antibody in washing solution should be tested (a typical dilution of detection antibodies might be in the range of 1:1000 to 1:10000).

Incubate the peptide array with diluted secondary antibody solution for 1-3 h in an incubation chamber (e.g. "4 Well Rectangular Dishes", NUNC can be used to incubate 4 arrays in parallel) on an orbital shaker or under a cover slip in a humidity chamber at room temperature. Remove the cover slip carefully by rinsing with blocking solution and wash the slides as described under ***2.3***.

2.5 Detection

Different detection methods can be used to visualize the spots that bound antibody or proteins on the surface of the array. Some of them are chemiluminescence, enzymatic color development or autoradiography.

2.5.1 Chemiluminescence (HRP labeled secondary antibody)

For chemiluminescence different detection kits are on the market. One example for a sensitive kit is the ECL Advance Western Blotting Detection Kit (product code RPN2135, Amersham Biosciences).

2.5.2 Enzymatic color development

Enzymatic color development can be done by using an AP labeled secondary antibody in combination with NBT/BCIP as substrate. Other conjugates and substrates can be used as well (e.g. peroxidase and DAB substrate).

Typical protocol (short version):

Depending on the secondary antibody used for detection, TBS / TBS-T or PBS / PBS-T buffers should be used. For AP labeled antibodies use TBS / TBS-T buffers.

1) Immerse the peptide array in blocking solution for 1-4 h
2) Incubate the peptide array with diluted antibody or protein solution for 3-8 h in an incubation chamber on an orbital shaker at room temperature or over night at 4°C.
3) Briefly rinse the slide one time 30 sec with TBS-T or PBS-T, followed by three washing steps in TBS-T or PBS-T, 5 min each.
4) Incubate the peptide array with diluted secondary antibody solution for 1-3 h at room temperature.
5) Briefly rinse the slide one time 30 sec with TBS-T or PBS-T, followed by three washing steps in TBS-T or PBS-T, 5 min each.
6) Detection – please use the description of the kit use for detection.

Examples for chemiluminescence and DAB as colorimetric substrate:

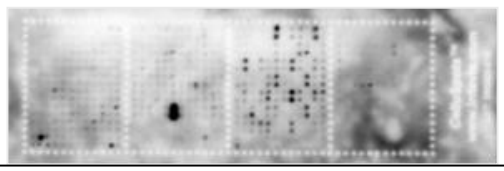


Fig 1: Peptide library, phospho-specific antibody incubation, detection: chemiluminescence (ECL kit).

Fig 2: Various borrelia antigens, serum incubation, detection: color development (DAB).

3 *Buffers and solutions*

3.1 Buffers

10 x Phosphate buffered saline (PBS), pH 7.4

- Dissolve 80 g NaCl, 2 g KCl, 26.8 g $Na_2HPO_4 \cdot 7H_2O$ and 2.4 g KH_2PO_4 in 800 ml H_2O
- Adjust pH to 7.4 with HCl
- Adjust volume to 1000 ml with H_2O
- Sterilize by autoclaving
- Store at room temperature

1 x Tris buffered saline (TBS), pH 7.5

- Dissolve 6.05 g Tris and 8.76 g NaCl in 800 ml H_2O
- Adjust pH to 7.5 with 1 M HCl
- Adjust volume to 1000 ml with H_2O
- Store at 4°C

3.2 Washing solutions

PBS-T: Dissolve 0.5 ml Tween-20 in 1000 ml PBS buffer.
TBS-T: Dissolve 0.5 ml Tween-20 in 1000 ml TBS buffer.

Note: Tween-20 is suitable for most applications. Depending on the antibodies or proteins used, different concentrations (between 0.01 and 1%) and/or detergents like SDS, Triton X or Nonidet P40 may lead to better results.

3.3 Blocking solutions

Different blocking solutions are described in the literature. For most applications a solution of 2 to 5% (w/v) skim milk powder in TBS-T or PBS-T is sufficient. If the background is high, alternative blocking solutions with BSA or casein can be used.