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1. Components

1.1 PEPperCHIP® Peptide Microarray

PEPperCHIP® Peptide Microarrays are provided on conventional glass slides (3" x 1", 75.4 mm x 25.0 mm x 1 mm). Besides standard or custom peptide content, we implement HA and FLAG peptides as internal controls and print spot duplicates. Depending on the size of the array, up to 16 array copies per slide can be printed. Sub-arrays can be separately stained and analyzed using the PEPperCHIP® Incubation Tray.

1.2 Storage

PEPperCHIP®s should be stored at 4°C to ensure a long shelf life. If handled with care, chips are stable for months.

1.3 Additional material to be supplied by user

Buffers: (We recommend filtering the PBS buffer using a 0.45 µm filter before adding any supplements.)

1. Standard buffer: PBS, pH 7.4, 0.05% Tween 20
2. Blocking buffer: PBS, pH 7.4, 0.05% Tween 20 with 1% BSA, or neat Rockland Blocking Buffer MB-070
3. Staining buffer: PBS, pH 7.4, 0.05% Tween 20 with 0.1% BSA or 10% Rockland Blocking Buffer MB-070

Equipment:

1. Incubation Tray: We recommend using the PEPperCHIP® Incubation Tray to work with minimal sample volumes and separately stain multiple array copies per slide. The PEPperCHIP® Incubation Tray is available in different array layouts. The PEPperCHIP® Peptide Microarrays are also compatible with the TECAN Hyb Station.
2. Orbital shaking device
3. Microarray scanner/reader: Depending on the labeling of secondary and control antibodies, read-out can be done with either fluorescence scanners or plate readers with a resolution of 21 µm or better. Detailed protocols depend on the scanner and/or plate reader and should be available from the manufacturer. The PEPperCHIP® Peptide Microarrays are compatible with common microarray scanners (e.g. GenePix 4000B, 4100B and 4300/4400 Microarray Scanners, Tecan Laser and Power Scanner, Agilent High-Resolution and SureScan Microarray Scanner, Innopsys InnoScan 710 and 900, NimbleGen MS 200 Microarray Scanner, LI-COR Odyssey Imaging System and many more).

Antibodies:

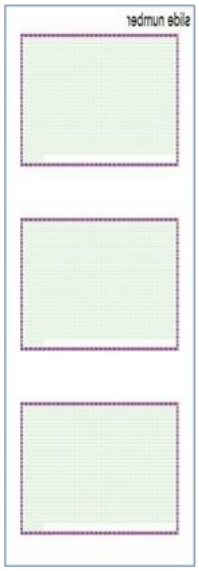
1. Primary sample (e.g. antibodies or sera)
2. Labeled secondary antibody for staining of primary sample (e.g. mouse anti-human antibody): Please note that strongly charged fluorescence dyes can interact with acidic and basic side chains of peptides. We, thus, recommend the use of neutral dyes or dyes with few charged functional groups (e.g. DyLight 680, DyLight 800, Cy3, Cy5), as well as pre-incubation with secondary antibodies to screen for background interactions. PEPperPRINT uses Pierce infrared dyes and the LI-COR Odyssey® Infrared Imaging System.
3. Labeled control antibodies, e.g. monoclonal anti-HA (12CA5)-DyLight 680 and monoclonal anti-Flag(M2)-DyLight 800 or monoclonal anti-HA (12CA5)-Cy5 and monoclonal anti-FLAG(M2)-Cy3 antibodies (see also PEPperCHIP® Staining Kit)

2. Staining protocols

2.1 Overview about array handling

Please have a look on the following pages concerning PEPperCHIP® handling.

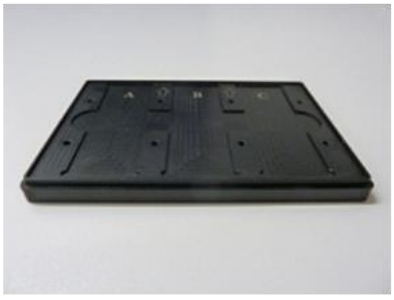
1. Each PEPperCHIP® is marked with an individual number on the backside of the slide. The slide is placed correctly in an incubation tray with the microarray surface up when the slide number appears in the upper right corner in a mirror view manner.



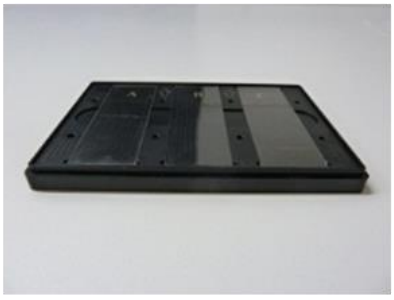
Samples, control and secondary antibodies must be applied to this PEPperCHIP® face.

Dust or other microscopic contaminations should be avoided as well since they can cause artifacts during the chip readout. Always handle peptide microarrays with care and wear laboratory gloves. PEPperCHIP®s should be touched with care only at the edges of the glass slide.

2. Place the basic plate of the PEPperCHIP® Incubation Tray in front of you.



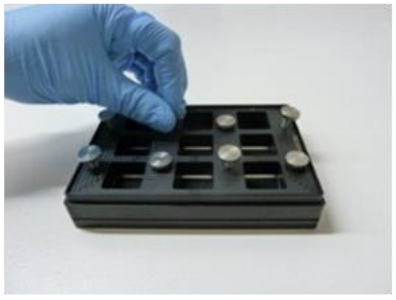
3. Place the array in the designated cavities of the base plate. Fill empty slide holders with dummy slides to prevent breaking of the array slide.



4. Place the seal with the glossy side facing downwards onto the slides.

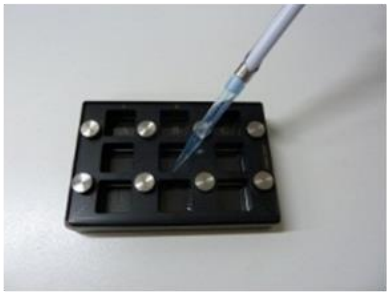
5. Place the upper part, which contains the thumbscrews, onto the seal.

6. Tighten alternated thumbscrews one after another by hand, starting from upper left, followed by lower right etc.. Do not fasten too tight.



7. You are finished if all thumbscrews are equally tightened.

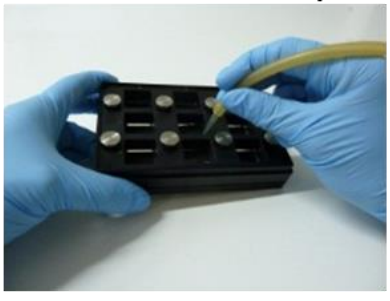
8. Slowly add buffer and staining solutions in the corner of an array chamber. Do not add it directly onto the array area.



9. Add the lid to avoid liquid evaporation.

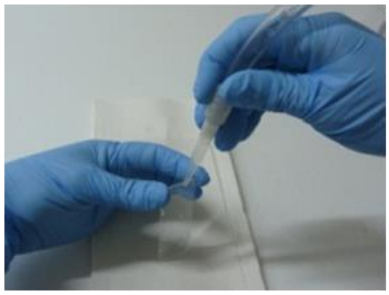
10. Use an orbital shaker with 250 rpm to get optimal wetting and staining conditions.

11. Aspirate liquid with a sucking device or pipette from the corner of the array chamber.

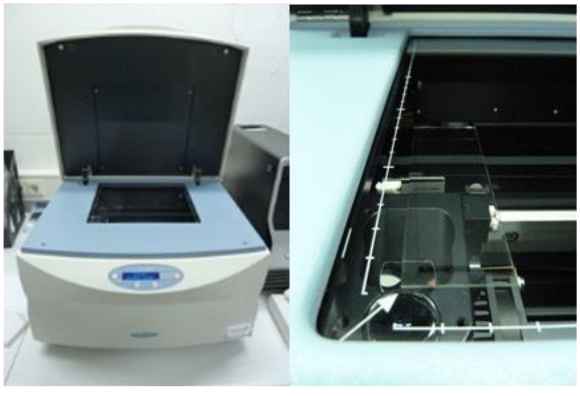


12. Before scanning, dip the slide into deionized water.

13. Dry the slide in an air stream blowing from top to bottom.



14. Scan the array image using a microarray scanner or reader. Place the slide onto the scanning device with the slide number in the lower left corner in a mirror view manner.



2.2 Recommended incubation volumes

The PEPperCHIP® Incubation Tray allows working with minimal sample volumes and staining of three chips in parallel. It is available in different layouts suitable for either staining of a full chip or separate staining of two, three, four, five or 16 array copies per chip. In the following table recommended incubation volumes are listed:

PEPperCHIP® Incubation Tray format	Incubation volume per well
3 x 1 well	1000 µl
3 x 2 wells	1000 µl
3 x 3 wells	500 µl
3 x 4 wells	500 µl
3 x 5 wells	400 µl
3 x 16 wells	100 µl

2.3 Pre-staining with secondary antibodies

Secondary antibodies used for staining of the primary sample can sometimes interact with peptide probes on the chip. To discriminate such background interactions from sample-specific signals, we recommend a pre-incubation and analysis of the PEPperCHIP® with secondary antibodies. This can be done either on the array used for sample incubation or any array copy, if available.

1. Incubate the PEPperCHIP® for 10 min at room temperature in standard buffer.

   Incubate the PEPperCHIP® with shaking (200 rpm) for 30 - 60 min at room temperature in blocking buffer to reduce non-specific interactions with the sample or secondary antibodies.

   Please note that shaking incubation significantly improves sample circulation to avoid gradients that may cause a bias in microarray data.[1] We therefore recommend an orbital shaker at 200 rpm. Rocking incubation, however, may cause dewetting of the microarray surface and should be avoided.

2. Wash 1 min with slight shaking (200 rpm) in standard buffer.
3. Dilute the secondary antibody in staining buffer. Depending on the nature of primary and secondary antibodies, the dilution may vary from 1 : 500 to 1 : 10,000. We are usually starting from 1:5000. Please note that higher concentrations can cause stronger background signals.
4. Incubate the PEPperCHIP® with slight shaking (200 rpm) for 30 min at room temperature with the secondary antibody diluted in staining buffer.
5. Wash 3x1 min at 200 rpm with standard buffer and dip the slide into deionized water.

[1] W. Kusnezow et al., *Optimal Design of Microarray Immunoassays to Compensate for Kinetic Limitations*, Mol. Cell. Prot. **5**:1681-1696 (2006).

6. Dry the PEPperCHIP® carefully in a stream of air.
7. Analyze the PEPperCHIP® in a microarray scanner or plate reader. Signals within the array have to be subtracted from signals arising from interactions with the primary sample.

2.4 Staining with primary samples

1. Incubate the PEPperCHIP® with slight shaking (200 rpm) for 10 min at room temperature in staining buffer.
2. Dilute the primary sample in staining buffer.

   Please note that depending on the nature of the sample and the interaction parameters the dilution may vary from 1 : 10 to 1 : 1,000 with 1 : 1,000 as recommended starting dilution. Low antibody titers in sera and reduced affinities of mono- and polyclonal antibodies require higher concentrations.
3. Incubate the PEPperCHIP® with slight shaking (200 rpm) overnight at 4°C with the primary sample diluted in staining buffer.
4. Wash 3x1 min at 200 rpm with standard buffer.

   Please note that washing times and repeats have to be adjusted with low affinity interactions and/or high off-rates. Vigorous washing may release antibodies and other proteins from the peptides and thus cause a loss in primary signals. Washing can be reduced down to 3x10 sec.

   If a PEPperCHIP incubation tray is used for one-by-one assays with multiple array copies per slide, we recommend to remove incubation and washing buffers by careful suction of the solutions e.g. by a pipette under slight tilting of the incubation tray. Please avoid contact of any tip with the microarray surface.

2.5 Staining with secondary antibodies

1. Dilute the secondary antibody in staining buffer.

   Please note that depending on the nature of primary and secondary antibodies, the dilution may vary from 1 : 500 to 1 : 10.000. We are usually starting from 1:5000. Please note that higher concentrations can cause stronger background signals.
2. Incubate the PEPperCHIP® with slight shaking (200 rpm) for 30 min at room temperature with the secondary antibody diluted in staining buffer.
3. Wash 3x1 min at 200 rpm with standard buffer and dip the slide into deionized water.

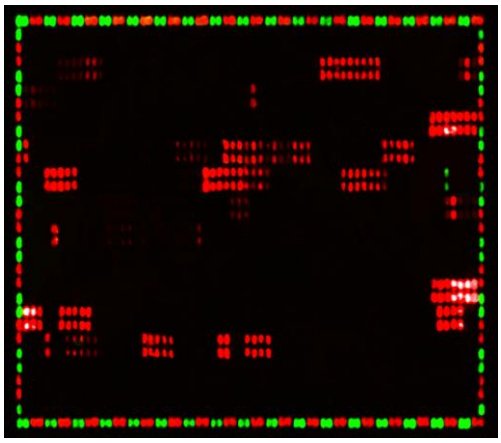
For final dipping into deionized water and drying we recommend removing the PEPperCHIP® from any incubation tray used.

4. Dry the PEPperCHIP® carefully in a stream of air.
5. Analyze the PEPperCHIP® in a microarray scanner or plate reader.

2.6 Staining with labeled control antibodies

1. Incubate the PEPperCHIP® for 10 min at 200 rpm at room temperature in staining buffer.
2. Dilute both control antibodies 1 : 1,000 together in staining buffer.
3. Incubate the PEPperCHIP® with slight shaking (200 rpm) for 1 h at room temperature with the control antibodies.
4. Wash 4x1 min at 200 rpm with standard buffer and dip the slide into deionized water.
5. Dry the PEPperCHIP® carefully in a stream of air.
6. Analyze the PEPperCHIP® in a microarray scanner or plate reader.

3. Read-out of chip experiments

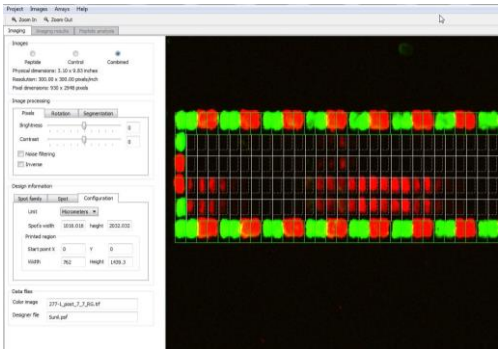


Place the slide onto the microarray scanner with the slide number in the upper right corner in a mirror view manner. Follow the manual of your scanner for image recording. Save the image as 16-bit grayscale TIFF image.

Epitope mapping of a polyclonal antibody sample against the antigen translated into overlapping 13mer peptides with 12 amino acids peptide-peptide overlap in duplicates. Each signal stretch represents an epitope that was bound by the antibody sample. Red and green stained HA and FLAG control peptides frame the antigen-derived peptides.

4. Data analysis

Each PEPperCHIP® is provided with GAL files for image analysis with GenePix® Pro, TIGR Spotfinder and other suited software solutions, as well as with Excel spreadsheets with the microarray layout for manual spot annotation. Please note that PEPperCHIP® spot morphologies are rectangular, what may require certain adjustments of spot geometry settings in GenePix® Pro and TIGR Spotfinder. We recommend usage of our own software solution **PepSlide® Analyzer** as cost-effective and intuitive alternative to GenePix® Pro. To acquaint yourself with the data analysis procedure you can start with a 30-days free trial version of PepSlide® Analyzer. Subsequently, an annual or a perpetual



version can be purchased. Each PEPperCHIP® is accompanied by a psf-file that assigns the peptide sequences to the spots. The psf-file is a grid that can be aligned to the raw scanner image by simply drag and drop. A readout file is created, which is fully compatible with Excel and summarizes for each spot the signal intensity, the background value and the corresponding peptide sequence. PEPSlide® Analyzer also helps to find consensus motifs (epitopes) within overlapping peptides and to decipher peptides with highest spot intensities of permutation scans.

5. Contact and support

For further questions and support, please don't hesitate to contact us by email or phone. We will try to be as supportive as possible. Please find our contact data below.