

## Protocol

# PepStar™ Microarrays

Ready-to-use peptide microarrays for antibody profiling

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## 1 Introduction

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Antibody/antigen interactions are a key event in immunology. Therefore, the identification of epitopes or immunodominant regions in antigens represents an important step in characterization of antibodies. One of the most efficient ways to identify such epitopes is incubation of a collection of antigen derived peptides displayed as peptide microarrays (PepStar™) with antibodies of interest.

JPT Peptide Technologies' PepStar™ peptide microarrays are customized peptide microarray sets for rapid screening of antibody peptide interaction displayed on glass slides. The peptides could represent overlapping peptides derived from the primary structure of antigens allowing extremely efficient epitope mapping of antibody collections directed against same antigens i.e. therapeutic antibodies. Alternatively, collections of peptide antigens like overlapping peptide scans through complete microbial or viral proteomes could be immobilized on the glass slides enabling characterization of general immune responses in blood serum. Upon incubation with your antibody or serum the binding event can be detected by reading the fluorescence intensity of either directly labeled primary antibody or secondary antibody directed against human IgG, respectively.

## 2 List of components

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Component	remark
PepStar peptide microarray	glass slides displaying peptides in triplicates
Blank slides engraved with "Dummy"	one blank slide per PepStar™ peptide microarray
Vials containing 20 spacers each	2 spacers per PepStar™ peptide microarray
Data CD-ROM	one CD-ROM including relevant files for specific peptide microarray (protocols as .pdf-file and sequence info as .gal-file)

## 3 Storage and Handling

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### 3.1 Storage of PepStar Peptide Microarray Slides

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- Optimal storage conditions for peptide microarray slides are in a cool (approx. 4°C / 39°F) and dry environment. Peptide microarrays are stable for at least 18 months when stored at 4°C (39°F).
- Do not freeze the peptide microarrays for prolonged storage.

### 3.2 Handling of PepStar Peptide Microarray Slides

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- Always handle the delicate peptide microarrays with care.
- Never touch the peptide microarray slide surface.
- Always wear laboratory gloves when handling peptide microarray slides.
- Please hold peptide microarray slides at the end, which carries the engraved data label. This label provides a unique identification of the array.
- Please take care when dispensing solutions onto the slide surface. Make sure not to touch the surface with pipette-tips or dispensers.
- Never whisk the surface of the peptide microarray slide with a cloth.
- Never use other chemicals as described. Inappropriate chemicals may destroy the chemical bonding of the peptides to the glass surface.
- Avoid dust or other particles during each step of the experiment. Dust, particles and resulting scratches will cause artifacts during the final signal readout.
- Please filter all solutions for the washing steps through 2µm, preferably 0.4µm particle filters before use.

**PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING YOUR EXPERIMENTS!  
CAREFULLY NOTE THE HANDLING AND STORAGE CONDITIONS OF PEPSTAR™  
PEPTIDE MICROARRAYS.**

**PLEASE CONTACT JPT PEPTIDE TECHNOLOGIES' TECHNICAL SERVICES FOR  
ASSISTANCE IF NECESSARY.**

## 4 Additional Materials Required

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### 4.1 Materials and Solutions

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- Blocking reagent (Pierce Biotechnology Inc. (#37536))
- Primary antibody/sera solution (JPT recommends a final antibody concentration of about 10-30µg/mL for primary antibodies; in case of human sera JPT recommends a dilution of 1:100 to 1:500)
- Fluorescently labelled secondary antibody stock solution (JPT recommends a final concentration of about 1µg/mL). For detection of human IgG JPT recommends Cy5-labelled antibody supplied by Jackson Immuno Research (order number: 209-175-082)
- TBS buffer (Tris buffered saline)
- Double distilled water for final washing steps of the microarrays

### 4.2 Additional Hardware and Software

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- Incubation/hybridisation station capable of washing and incubating slides in a temperature controlled environment (JPT recommends Tecan Hyb Station HS4X00), alternatively a microarray incubation sandwich can be used (please refer to point 6.1.2 for further details)
- Microarray centrifuge or access to a stream of nitrogen to dry the microarray slides
- Fluorescence scanner/imager capable of excitation of appropriate fluorophore moiety and with a pixel size of at least 10µm
- Software tool allowing the assignment of signal intensities to spots on the surface of the peptide microarrays by interpreting enclosed .gal-file

## 5 General considerations

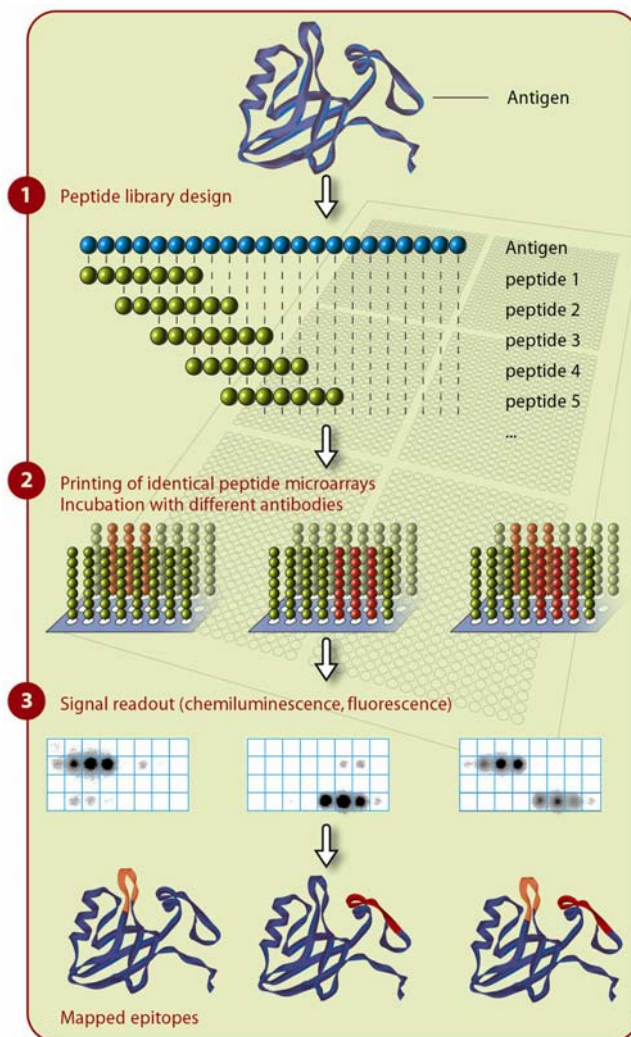
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### 5.1 Experimental basics

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JPT Peptide Technologies' PepStar™ peptide microarrays are comprised of peptides derived from different antigens (principle of epitope detection see Figure 1). The

deposited molecules represent either overlapping peptides derived from antigens or collections of known or potential antigenic peptides. An optimized hydrophilic linker moiety is inserted between the glass surface and the antigen derived peptide sequence to avoid false negatives caused by sterical hindrance. The density of the peptide molecules within each spot on the PepStar™ peptide microarray is 15fmol/mm<sup>2</sup> allowing very sensitive detection of bound antibodies. The peptides are displayed in three identical subarrays on each slide. PepStar™ slide surfaces are delivered in a pre-treated form minimizing unspecific binding of your target antibody. Therefore, no blocking step is needed. The data CD-ROM included with the PepStar™ peptide microarray contains all information needed for the detailed analysis of your data including peptide sequences as well as the position of the appropriate peptides on the glass surface by means of a .gal-file.



JPT's PepStar™ Peptide Microarrays are devices for detecting potential biomarkers for infectious diseases, autoimmune diseases, cancer and allergies. Each spot in the microarray represents a single individual peptide.

During incubation of the peptide microarray with patient serum bound antibodies could be detected using fluorescently labelled anti-human-IgG antibodies

Resulting antibody signatures represent unique fingerprint of immune status.

**Figure 1:** General principle of epitope detection using overlapping peptide scans

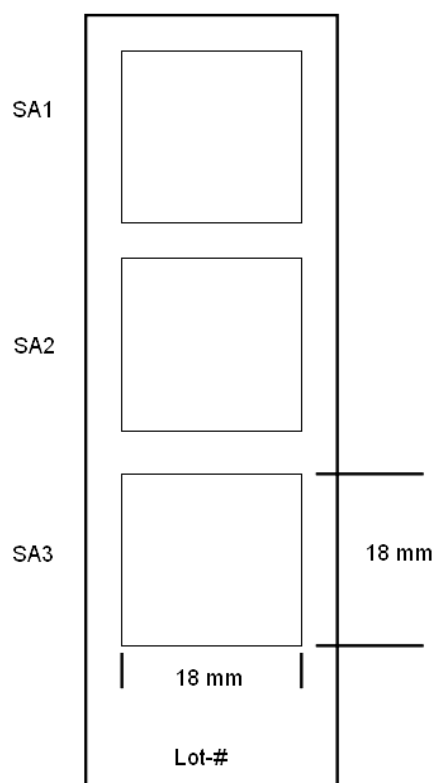
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## 5.2 PepStar™ peptide microarray Layout

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Please refer to the .gal-file on the enclosed CD-ROM for the identity and location of the spots on the microarray surface. The side of the slide with the engraved label represents the surface displaying the attached peptides. The .gal-file can be opened using microarray evaluation software-modules capable of evaluating high-density microarray slides. Since .gal-files are tab-separated text files, they can be processed with software modules such as Microsoft Editor (Notepad) or Microsoft Excel.

A schematic layout of the peptide microarray is shown in Figure 2.



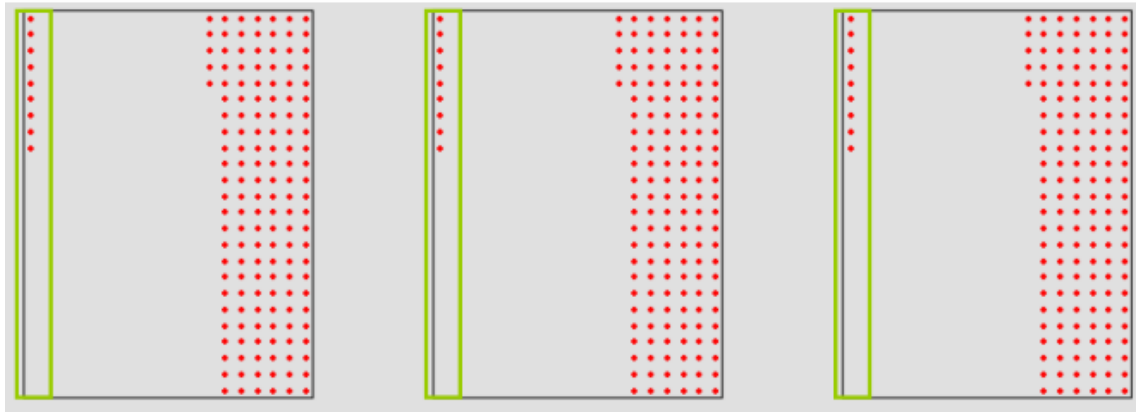
**Figure 2:** schematic layout of peptide microarray (SA=subarray)

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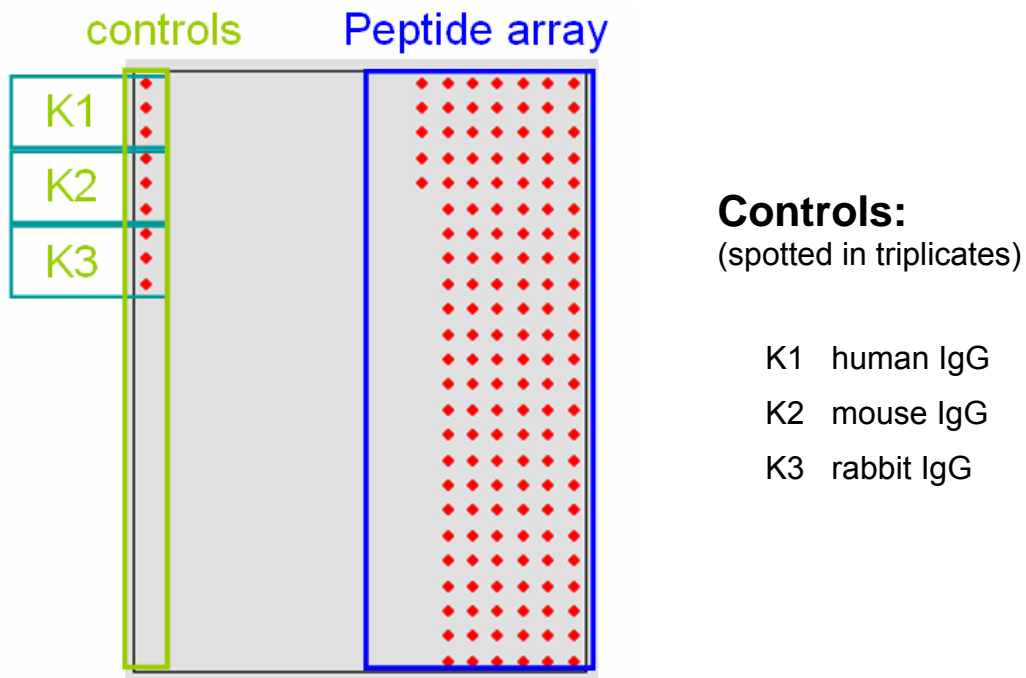
As shown in Figure 2 the peptide microarray is printed in three identical subarrays (SA). This enables efficient intra-chip-reproducibility tests using scatter plots or correlation functions.



For lower density peptide microarrays (number of Spots per subarray <384) or if larger spot diameters are requested, the peptides will be deposited in one block only. In that case, the layout will differ slightly to above shown figures. The principal layout of three subarrays enabling intra-chip reproducibility tests will still be valid (see Figure 5 and Figure 6).



**Figure 5:** General low density microarray layout, three subarrays are visible with vertical aligned positive controls (green frame)



**Figure 6:** Subarray layout, peptide array is framed blue (starting in the upper right corner); positive controls are vertical aligned on left-hand side

Independent if a peptide microarray is declared as high or low density peptide microarray, the .gal file will induce an appropriate grid for simple spot recognition and evaluation.

## 6 Experimental protocols

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**Note: The following procedure is given as guideline only. The optimal experimental conditions will vary depending on the investigated parameters and cannot be predetermined - they must therefore be established by the user. No warranty or guarantee of performance using this procedure with your target antibody or serum can be made or is implied.**

The PepStar™ peptide microarray is designed as a ready-to-use product to identify epitopes or immunodominant regions in antigens directly on the glass slide surface. There is no need to perform blocking steps on the slide surface prior to incubation with the target antibody or protein. However, in case of incubations with patient sera or plasma, JPT recommends to include an additional blocking step prior to incubation with patient sample (refer to point 4.1).

Please refer to the .gal-files on enclosed CD-ROM for the identity and location of the spots on the peptide microarray surface. The side of the glass slide with the engraved label represents the slide surface displaying the attached peptides.

### 6.1 General principles for incubation

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#### 6.1.1 Fully automated microarray processing station

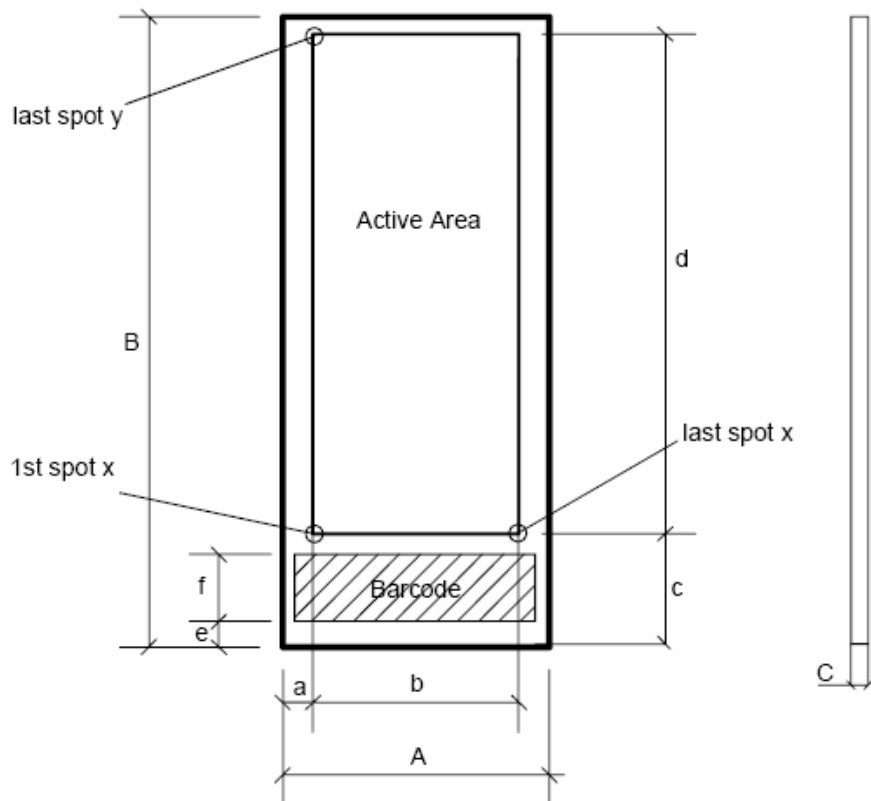
All peptide microarrays produced by JPT have an identical layout concerning active area and spotted surface. Although the content of the microarrays varies the overall layout and dimensions are the same (see Figure 7).



Please check with the manufacturer of your microarray processing station for compatibility with the required liquids. Most microarray processing stations are sensible towards strong acids and organic solutions. Protocols have to be adapted to prevent permanent damage to your device.

All peptide microarrays produced by JPT are adjusted to fit in common fully automated microarray processing systems. JPT recommends using Tecan HS4X00 Hybridisation systems.

Protocols and procedures for using Tecan HS4X00 systems can be provided by JPT if necessary.

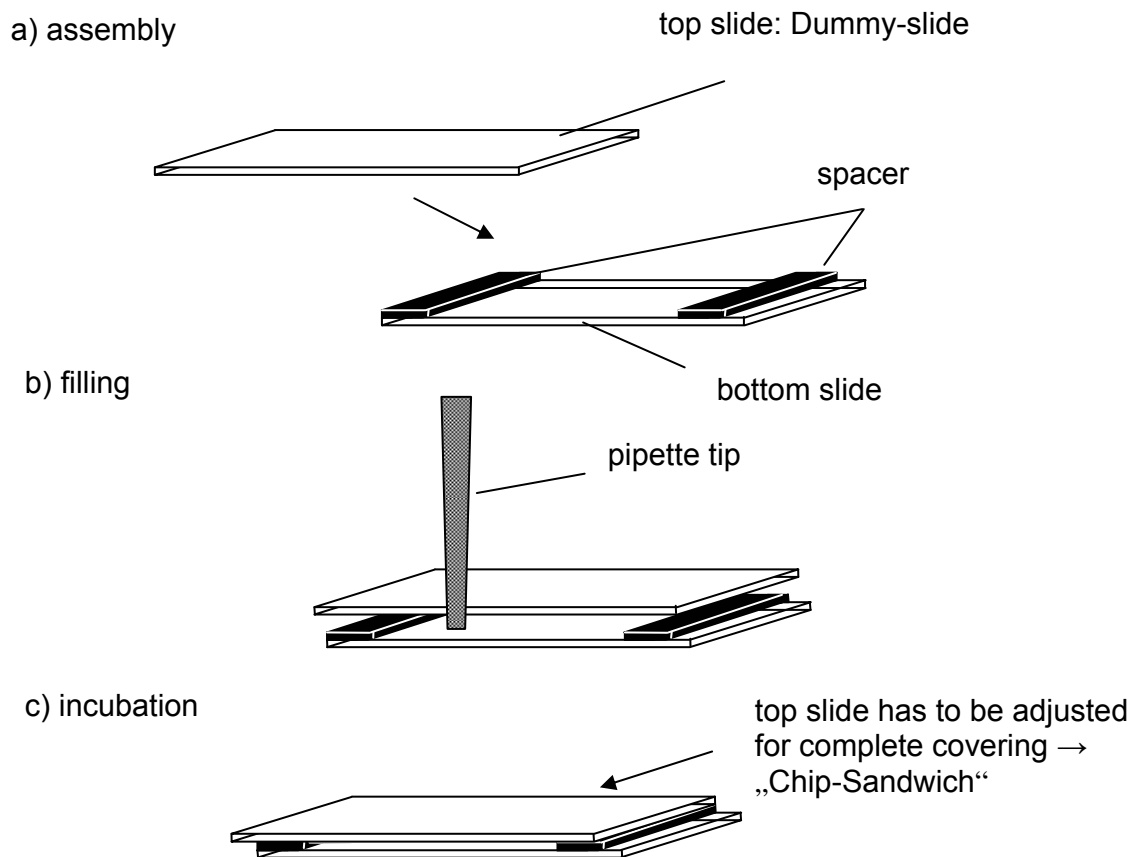


	Distance (mm)
A	25
B	75
C	1
a	3.5
b	18
c	9
d	57
e+f	1+8

**Figure 7:** Maximum area dimension on JPT peptide microarrays

## 6.1.2 Microarray incubation using microarray-chip-sandwich

To create a simple incubation chamber, two slides, one displaying the peptides and another slide (Dummy-slide) without any peptides, have to be assembled according to Figure 8 in a sandwich like format. If two peptide microarrays should be screened the top slide could be another peptide-displaying chip. Please make sure that in such a case the two peptide-displaying sides are facing each other. The two slides are separated by two spacers.



**Figure 8:**

- a) Assembly of “Chip-Sandwich” is shown. Two plastic spacers are placed between the peptide displaying microarray (bottom slide) and the Dummy-slide or second peptide displaying microarray (top slide) resulting in a defined reaction chamber.
- b) Assay solution is applied via pipette tip into the reaction chamber formed by the two slides. Capillary forces will soak-in the solution without formation of bubbles.
- c) top microarray is shifted resulting in overlaying ends of the glass slides. This arrangement enables convenient disassembly after the incubation step.

The final assay volume will depend on the thickness of the plastic spacers (enclosed spacers show a thickness of approx 200µm, which will result in a total volume of 250

to 300 $\mu$ L in between the two slides). The sample has to be applied in between the two slides. Therefore, the top slide is shifted about 1mm to one side. If the pipette tip is adjusted on the position directly over the uncovered bottom slide the capillary forces allow proper distribution of the sample solution without formation of bubbles.

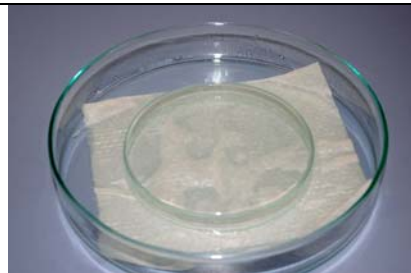
After the incubation is finished, open the microarray sandwich in TBS-buffer, remove the plastic spacers and rinse the peptide microarrays thoroughly with TBS-buffer before continuing with the assay protocol.



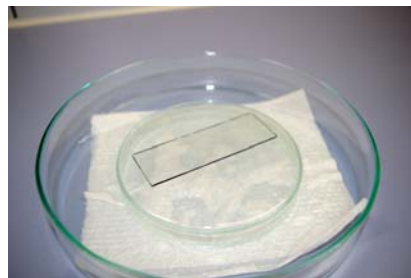
JPT does not recommend use of fluorescently labelled primary or secondary antibodies in microarray sandwich-like incubations. Instead microarrays should be washed in solutions containing fluorescently labelled antibodies since the resulting background will be decreased resulting in better signal-to-noise ratio.

### 6.1.2.1 Prepare the slide-environment for easy handling

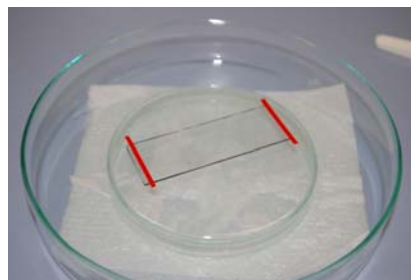
- I. Place a small Petri-dish upside down into larger Petri-dish to serve as support plate for the microarray. Place a piece of wet cloth underneath the small Petri-dish. This will keep the incubation solution from evaporating once the large Petri-dish is closed.



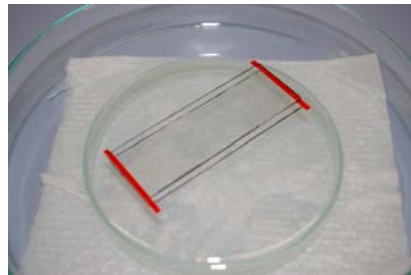
- II. Place the peptide microarray slide support plate facing upward (engraved label has to be readable from top).



- III. Place enclosed spacers on both ends of the microarray.

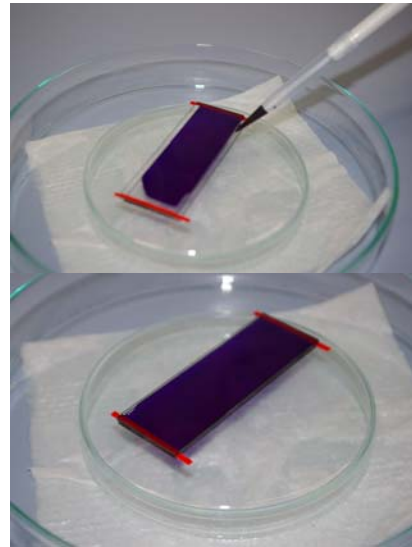


- IV. Prepare the microarray sandwich according to description above (6.1.2). If two peptide microarrays are used make sure that peptide displaying sides are facing each other.



- V. Prepare approx. 300 $\mu$ L of final assay solution (if enclosed spacer are used) containing your target antibody/sera.

- VI. Pipette the complete volume into microarray chip sandwich. Capillary forces will suck the solution in between the two slides. Make sure there are no air bubbles within the sandwich.

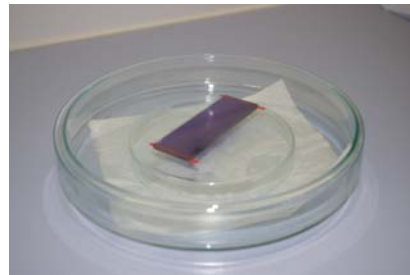


- VII. Adjust the peptide microarray sandwich as described in point 6.1.2 and Figure 8.



Make sure not to touch the microarray with the pipette tip. Scratches and marks on the surface may destroy the deposited microarray and will cause artefacts!

- VIII. Close the Petri-dish with a matching cover to create an incubation chamber.



### 6.1.2.2 Incubation with primary antibody / sera

- IX. Incubate the peptide microarray for the appropriate time and temperature with your antibody/sera. JPT recommends an incubation time of at least 2 hours at approximately 30°C (86°F).

### 6.1.2.3 Wash microarray

- X. Disassemble microarray sandwich and wash the slide 5 times with TBS-buffer (3-4min each wash) to remove excess antibody. Ensure that microarray is properly washed with enough liquid rinsing over the slide.

#### **6.1.2.4 Incubation with secondary antibody**

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- XI. Incubate the peptide microarray for the appropriate time and temperature with your fluorescently labelled secondary antibody. JPT recommends an antibody concentration of 1µg/mL and an incubation time of at least 30 minutes at approximately 30°C (86°F).

#### **6.1.2.5 Wash microarray**

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- XII. Wash the slide 5 times with TBS-buffer (3-4min each wash) to remove excess antibody. Ensure that microarray is properly washed with enough liquid rinsing over the slide.
- XIII. Wash the slide 5 times with de-ionized water (3-4min each wash) in order to remove all salt residues. Ensure that the slide is properly washed with enough liquid rinsing over the slide.
- XIV. Dry the slides using a microarray centrifuge or by blowing a gentle stream of nitrogen on the microarray surface.

#### **6.1.2.6 Imaging of PepStar peptide microarray**

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- XV. Perform fluorescence scans according to your scanner type and with laser settings corresponding to the fluorescence label of the secondary antibody. We recommend a resolution with a pixel size of at least 10µm.

#### **6.1.2.7 Data analysis**

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- XVI. Generate a list containing signal intensities of each peptide spot.
- XVII. Calculate the mean value for the signal intensities of spots with identical peptides (three identical subarrays). JPT recommends using background corrected values for final signal intensity.
- XVIII. Arrange the results according to the mean value. Start with highest value. The highest values indicate the spots displaying peptides recognized most effectively by your antibody.

**For details about application and modification of .gal files, please refer to the protocol: "reading a \_gal-file"**

## 7 Notes

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- Fluorescence scanning could be very sensitive depending on the scanner. Avoid any fluorescent impurities/contaminations inside your assay solution or wash solutions. You can easily check for such impurities incubation and washing a Dummy-slide with the same solutions followed by imaging.
- Blocking with protein containing solutions like bovine serum albumin (BSA) can cause depending on the source and preparation high background signals impairing the final results. If you need such a blocking step (not recommended by JPT) please scan the PepStar™ peptide microarray subsequent to this blocking step and use that image as a starting point for your analysis.
- Avoid dust or other particles during each step of the experiment. Dust particles and resulting scratches will cause artefacts during the final signal readout.
- For incubation with the fluorescently labelled secondary antibody it is important to use metal trays with a cover or plastic trays completely covered with aluminium foil as these antibodies are sensitive towards light.
- Control incubations using labelled secondary antibody alone should be performed in parallel to the epitope mapping experiment to ensure that found signals are not caused by unspecific binding of the secondary antibody to the immobilized peptides.
- Carefully adjust the final dilution of your labelled secondary antibody. Microarray technology is very sensitive and therefore it could be possible to use the secondary antibody in a higher dilution as proposed by the manufacturer. Generally, 1:1000 dilutions of a 1mg/mL stock solution are working very well. Nevertheless, depending on the nature of the secondary antibody, such concentrations may yield high background signals caused by unspecific binding to the coated glass surface. If the signals within the peptide spots are high, you could test 1:5000 or 1:30000 dilutions of a 1mg/mL stock as well.

## 8 Related products

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For further information visit our homepage ([www.jpt.com](http://www.jpt.com)) or contact our customer support.

- PepSpots™: customized peptide arrays on cellulose membranes