

## Protocol

# RepliTope™ Antigen Collection Microarrays

Ready-to-use peptide microarrays for antibody profiling

### Contact us:

InfoLine: +49-30-6392-7878  
Order per fax: +49-30-6392-7888  
Or e-mail: [peptide@jpt.com](mailto:peptide@jpt.com)  
www: [www.jpt.com](http://www.jpt.com)

JPT Peptide Technologies GmbH  
Volmerstrasse 5 (UTZ)  
12489 Berlin  
GERMANY

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## 1 Introduction

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

JPT Peptide Technologies' RepliTope™ antigen collection peptide microarrays represent catalog peptide microarray slides for rapid screening of protein-peptide interactions. The purified peptides displayed on glass slides are chemoselectively and covalently bound, enabling effective interaction with binding partners. Immobilized sequences represent overlapping peptides derived from multiple antigens allowing efficient profiling of humoral immune responses using patient samples and protein-protein interaction studies. In addition, full proteome RepliTope™ are available that cover overlapping peptide scans through complete microbial or viral proteomes. Upon incubation with your protein or patient sample the binding event can be detected by reading out photon intensity emanating from the fluorescently labeled primary or secondary antibody.

## 2 List of Components

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Component	Remark
RepliTope™ peptide microarray	Glass slide displaying peptides
Blank slides engraved with "Dummy"	One blank slide per RepliTope™ peptide microarray
Vials containing 20 spacers each	2 spacers per RepliTope™ peptide microarray
Data CD-ROM	One CD-ROM including relevant files for the specific peptide microarray (protocols as .pdf-file, sequence info as .gal-file and GalViewer software)

## 3 Storage and Handling

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### 3.1 Storage of RepliTope™ 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 6 months when stored at 4°C (39°F).
- Do **not** freeze the peptide microarrays for prolonged storage.

### 3.2 Handling of RepliTope™ 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 specific microarray.
- 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 chemicals other than 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 final signal readout.
- Please filter all solutions for the washing steps with minimum 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 REPLITOPE™  
PEPTIDE MICROARRAYS.**

**PLEASE CONTACT JPT PEPTIDE TECHNOLOGIES' TECHNICAL SERVICE FOR  
ASSISTANCE OR FOR QUESTIONS.**

## 4 Additional Material Required

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### 4.1 Material and Solutions

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- Blocking reagent (Pierce Biotechnology Inc. (#37536))
- Primary antibody/sera solution (JPT recommends a final antibody concentration of about 1-10 $\mu$ g/mL for primary antibodies; in case of human sera JPT recommends a dilution of 1:100 to 1:500)
- Fluorescently labeled secondary antibody stock solution (JPT recommends a final concentration of about 1 $\mu$ g/mL). For detection of human IgG JPT recommends Cy5-labeled 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

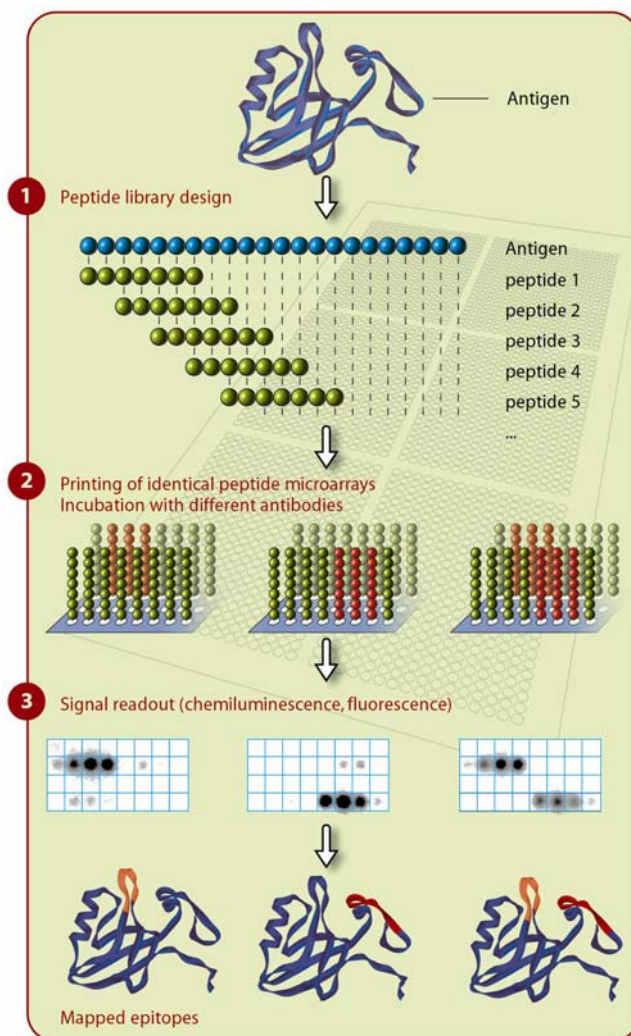
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- Incubation/hybridisation station capable of washing and incubating slides in a temperature controlled environment (JPT recommends Tecan Hyb Station HS4X00). Alternatively a microarray incubation sandwich can be used (please refer to point 6.1.2 for 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 10 $\mu$ m
- 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

### 5.1 Experimental Basics

JPT Peptide Technologies' RepliTope™ antigen collection peptide microarrays comprise purified synthetic peptides derived from antigens (principle of epitope detection see Figure 1) or other sources that are chemoselectively and covalently immobilized to the glass surface. 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. For technical reasons all peptides contain a C-terminal glycine.



JPT's RepliTope™ antigen collection peptide microarrays are designed for detecting potential biomarkers for infectious diseases, autoimmune diseases, cancer and allergies and to elucidate protein-protein interactions. Each spot in the microarray represents a single individual peptide.

After incubation of the peptide microarray with human patient serum, bound antibodies can be detected using fluorescently labeled anti-human-IgG antibodies

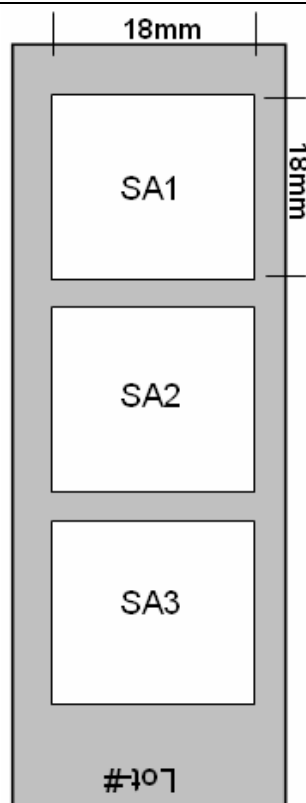
Resulting antibody signatures represent unique insights into the individual humoral immune status.

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

All peptides are displayed in three identical subarrays on each slide. RepliTope™ slide surfaces are delivered in a pre-treated form minimizing unspecific binding of your target protein. The data CD-ROM delivered with the RepliTope™ antigen collection peptide microarray contains all information needed for detailed analysis of your data, including peptide sequences and positions on the glass surface by means of a .gal-file.

## 5.2 RepliTope™ Peptide Microarray Layout

Please refer to the .gal-file on enclosed CD-ROM for identity and location of the spots on the microarray surface. The microarray side carrying 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 or the GalViewer-software enclosed on the CD-ROM. Since .gal-files are tab-separated text files, they can also 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 a peptide microarray (SA=subarray).

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

Each peptide is printed one time per subarray. With the .gal-file provided, evaluation can be performed using software modules like GenePix, ArrayPro or similar programs, which align the .gal-file induced grid onto the resulting image. JPT's GalViewer software can be applied for qualitative analysis and spot identification.

For RepliTope™ antigen collection microarrays, each subarray (SA) is printed in individual blocks (see Figure 3, details in Figure 4). The number of blocks and the final layout will vary according to the final number of peptides derived from the antigens.

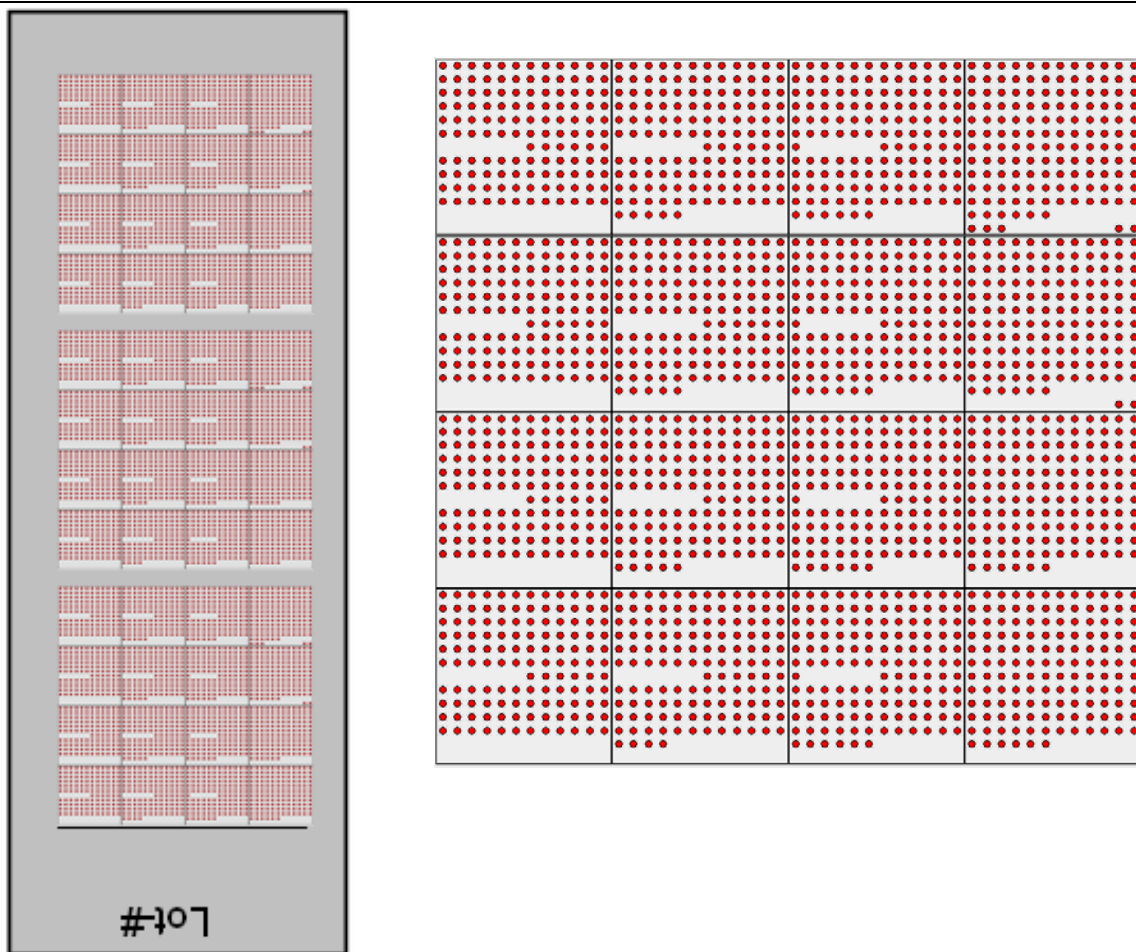


Figure 3: Exemplary view for a microarray slide with 3 subarrays (left) and a subarray consisting of 16 individual blocks (right).

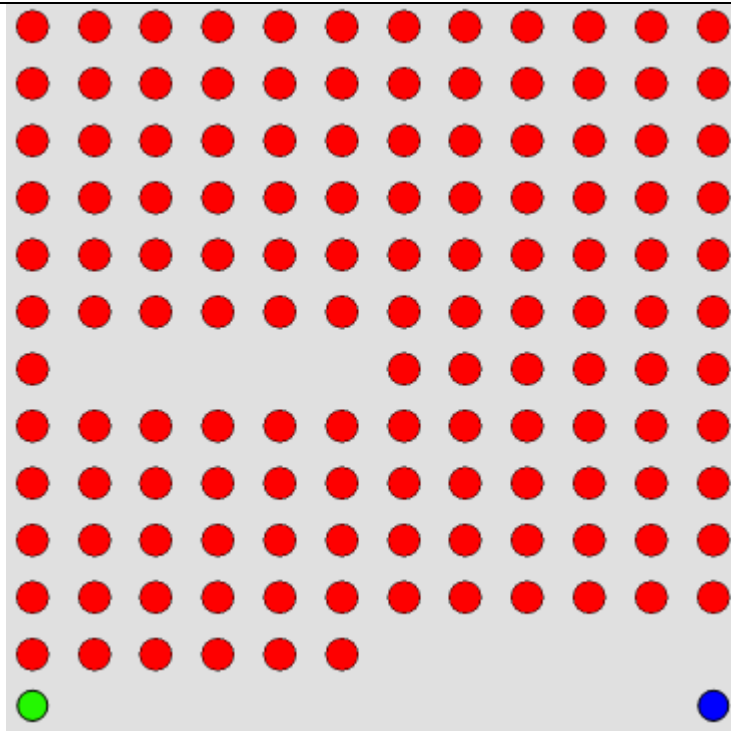


Figure 4: Exemplary block layout

Each spot represents an individual peptide. Green spot refers to process control (flag-tag-peptide), blue spot to fluorescent landmark (printed only in selected blocks, for details please refer to .gal-file). Blank spots between individual antigen scans are devoid of any peptide.

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## 6 Experimental Protocols

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

The RepliTope™ peptide microarray is designed as a ready-to-use product to identify epitopes, peptide binders or immunodominant regions in antigens directly on the glass slide surface. In case of seroscreening applications, JPT recommends including blocking steps based on previous ELISA experiments prior to incubation. If you would like to perform an additional blocking reaction, please use recommended blocking reagents (refer to point 4.1).

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

## 6.1 General Principles for Incubation

### 6.1.1 Incubation using a 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 5).



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 into common fully automated microarray processing systems. JPT recommends using Tecan HS4X00 Hybridisation systems. All RepliTope™ microarrays are printed according to the layout of the Single chamber option of Tecans HS4X00 Pro station.

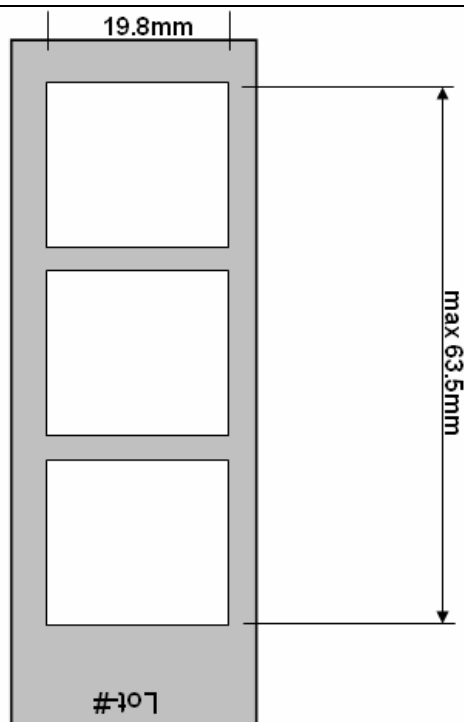


Figure 5: Maximum area dimension on JPT peptide microarrays.

An exemplary protocol for the use of JPT's RepliTope™ peptide microarrays in Tecan's HS4X00 processing machine is shown **Figure 6**.

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- W** 1 WASH: Temp. °C: 30.0, First: Yes, Ch.: 1, Runs: 3, Wash time: 0:02:00, Soak time: 0:03:00
  - P** 2 PROBE INJECTION: Temp. °C: 30.0
  - H** 3 HYBRIDIZATION: Temp. °C: 30.0, Agitation Frequency: Medium, Time: 0:30:00
  - W** 4 WASH: Temp. °C: 30.0, First: No, Ch.: 1, Runs: 2, Wash time: 0:02:30, Soak time: 0:02:30
  - P** 5 PROBE INJECTION: Temp. °C: 30.0
  - H** 6 HYBRIDIZATION: Temp. °C: 30.0, Agitation Frequency: High, Time: 2:00:00
  - W** 7 WASH: Temp. °C: 30.0, First: No, Ch.: 2, Runs: 3, Wash time: 0:02:30, Soak time: 0:00:30
  - P** 8 PROBE INJECTION: Temp. °C: 30.0
  - H** 9 HYBRIDIZATION: Temp. °C: 30.0, Agitation Frequency: High, Time: 0:45:00
  - W** 10 WASH: Temp. °C: 30.0, First: No, Ch.: 2, Runs: 5, Wash time: 0:02:00, Soak time: 0:00:00
  - W** 11 WASH: Temp. °C: 30.0, First: No, Ch.: 5, Runs: 1, Wash time: 0:02:30, Soak time: 0:01:30
  - S** 12 SLIDE DRYING: Temp. °C: 30.0, Time: 0:04:00, Final Manifold Cleaning: No, Ch.: No

Figure 6: Exemplary method for incubation of JPT's RepliTope™ microarrays in Tecan HS4X00 processing machines:

Ch.: 1 and 2: TBS buffer, 0.1% Tween20

Ch.: 5: 0.1x SSC buffer

Step 1: Pre-wash and filling of incubation chambers

Step 2-4: Blocking procedure

Step 5-7: Incubation with primary antibody/sera

Step 8-10: Incubation with secondary antibody

Step 11: Final washing steps

Step 12: Slide drying procedure

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## 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 7 in a sandwich-like format. If two peptide microarrays are to be screened in parallel, the top slide would 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.

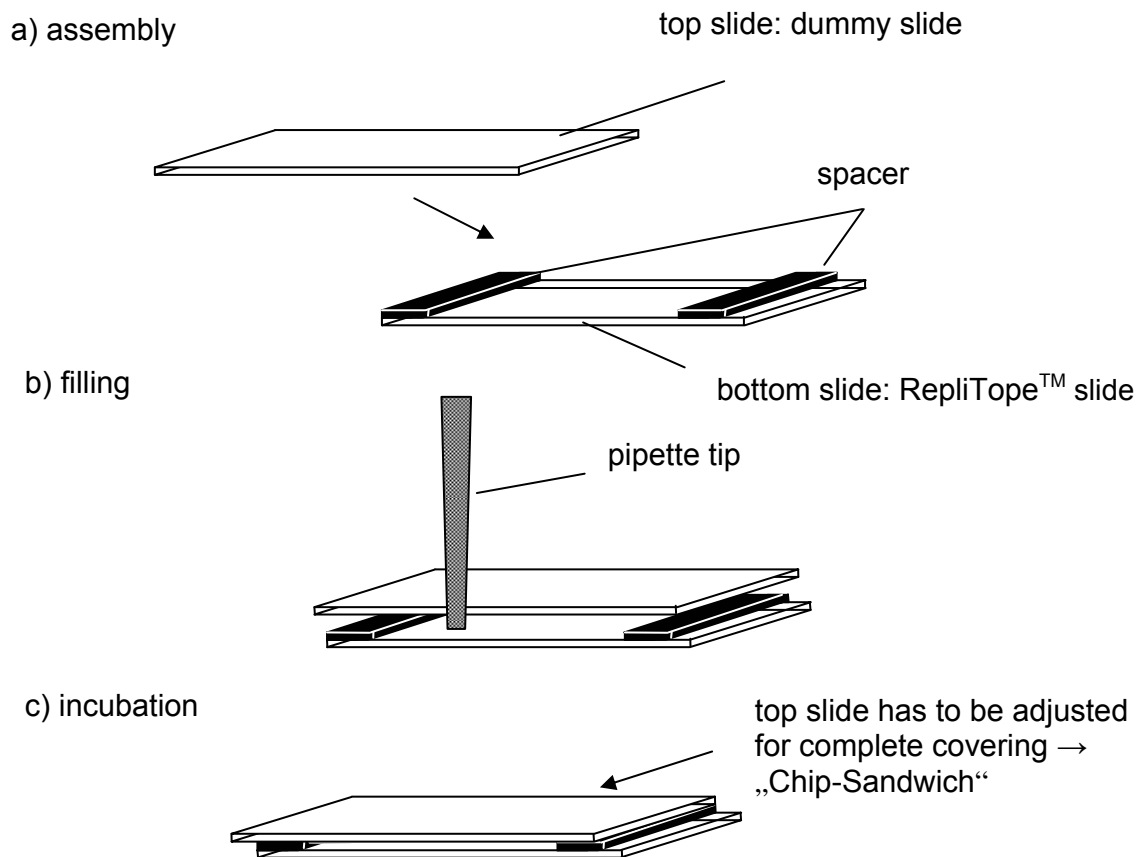


Figure 7: Assembly of a chip sandwich.

a) 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 overlapping 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 300

to 350 $\mu$ L in between the two slides). The sample has to be applied between the two slides. For that end, the top slide is shifted about 1mm to one side. If the pipette tip is adjusted to a position directly over the uncovered bottom slide the capillary forces allow proper distribution of the sample solution without formation of bubbles.

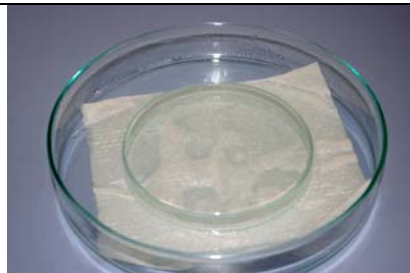
After the incubation is finished, open the microarray sandwich in TBS-buffer, remove the plastic spacers and rinse the peptide microarrays thoroughly with TBS-buffer before continuing with the assay protocol.



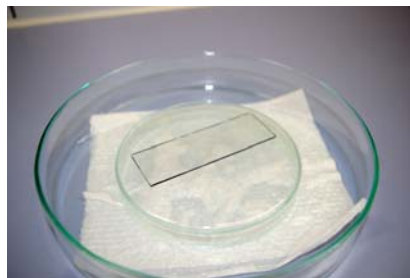
JPT does not recommend use of fluorescently labeled primary or secondary antibodies in microarray sandwich-like incubations. Instead microarrays should be washed in solutions containing fluorescently labeled antibodies since the resulting background will be decreased resulting in better signal-to-noise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

### 6.1.2.1 Prepare the slide environment for easy handling

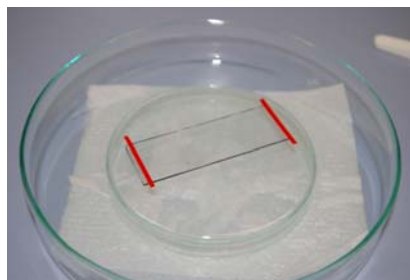
- I. Place a small Petri-dish upside down into larger Petri-dish to serve as support plate for the microarray. Place a piece of wet cloth underneath the small Petri-dish. This will keep the incubation solution from evaporating once the large Petri-dish is closed.



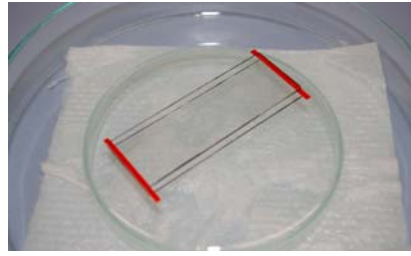
- II. Place the peptide microarray slide support plate facing upward (engraved label has to be readable from top).



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

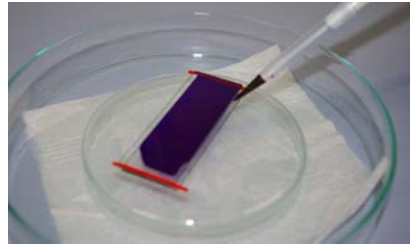


- IV. Prepare the microarray sandwich according to description above (6.1.2). If two peptide microarrays are used make sure that peptide displaying sides are facing each other.

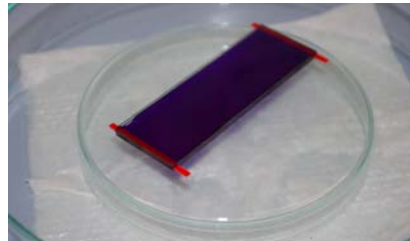


- V. Prepare approx. 400 $\mu$ L of final assay solution (if enclosed spacer are used) containing your target antibody/sera.

- 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.

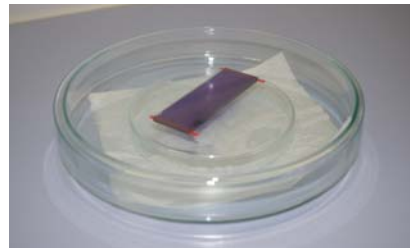


- VII. Adjust the peptide microarray sandwich as described in point 6.1.2 and Figure 7.



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 by putting the sandwich in a beaker filled with washing buffer and subsequently wash the Replotope microarray slide 5 times with TBS-buffer (3-4min each wash) in a petri dish to remove excess antibody.

#### **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 labeled secondary antibody. JPT recommends an antibody concentration of 0.1-1 µg/mL and an incubation time of at least 45 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 the microarray is properly washed with enough liquid rinsing the slide.
- XIII. Wash the slide 5 times with de-ionized water (3-4min each wash) in order to remove all buffer residues. Ensure that the slide is properly washed with enough liquid rinsing the slide.
- XIV. Dry the slides using a microarray centrifuge or by blowing a gentle stream of nitrogen onto the microarray surface.

#### **6.1.2.6 Imaging of RepliTope™ peptide microarrays**

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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 by means of a microarray evaluation software.
- XVII. Calculate the mean value for the signal intensities of spots with identical peptides (from 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" enclosed to the galviewer-software.**

## 7 Notes

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- Fluorescence scanning can be very sensitive depending on the scanner. Avoid any fluorescent impurities/contaminations within your assay and washing solutions. You can easily check for such impurities by incubating and washing a dummy slide with the same solutions followed by imaging.
- Blocking with protein containing solutions like bovine serum albumin (BSA) potentially causes high background signals impairing the final results. If you need such a blocking step, please scan the RepliTope™ peptide microarray subsequent to this blocking step but before incubation with your sample 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 labeled 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 labeled secondary antibody alone should be performed in parallel to the epitope mapping experiment to ensure that identified signals are not caused by unspecific binding of the secondary antibody to the immobilized peptides.
- Carefully adjust the final dilution of your labeled secondary antibody. Microarray technology is very sensitive and therefore it could be possible to use the secondary antibody in a higher dilution than 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 team.

- PepStar™: customized peptide microarrays displaying individually synthesized peptides in triplicates on one microarray
- PepSpots™: customized peptide arrays on cellulose membranes