Protocol

Multiwell Peptide Microarrays

Ready-to-use peptide microarrays for humoral immune response profiling and epitope mapping

Revision 1.2

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Table of Contents

1 Introduction

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 (peptide microarrays) with antibodies of interest.

JPT Peptide Technologies' peptide microarrays are ready-to-use 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 overlapping peptides derived from single or multiple antigens as well as epitope and random peptide collections allow efficient profiling of humoral immune responses using patient samples. In addition, the peptide microarrays can be used for protein-protein interaction studies. Upon incubation with your protein or patient sample the binding event can be detected by fluorescently labelled primary or secondary (2^{nd}) antibody.

2 List of Components

1. **Multiwell peptide microarray**

   Glass slide displaying peptides in 21 identical mini-arrays, printed in a pattern suitable for Multiwell-incubation chamber (also available from JPT), see section 4.3 for details

2. **Multiwell incubation chamber (if ordered separately)**

   Incubation chamber allowing parallel incubation of up to four microarray slides, enabling parallel assay of 84 individual samples, see section 4.3 for details

3. **Product information**

   Relevant files for the specific peptide microarray (protocol and datasheet as pdf-files, sequence info as gal-file and JPT's GalViewer software as zipped package)

3 Storage and Handling

3.1 Storage of Multiwell Peptide Microarray Slides

- 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!!!

3.2 Handling of Multiwell Peptide Microarray Slides

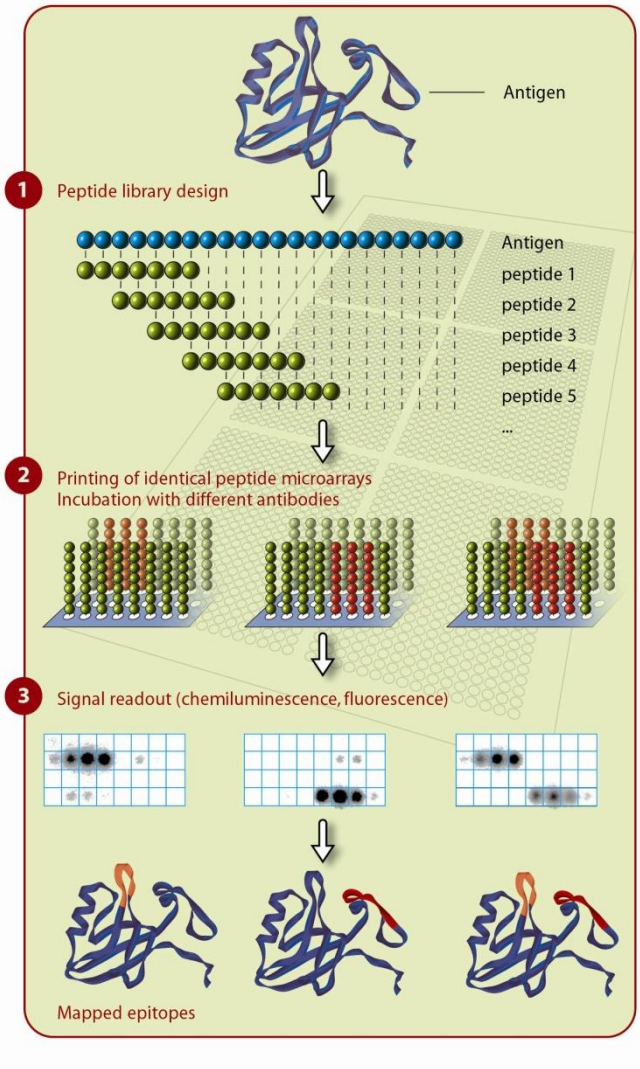
- Always handle the peptide microarrays with care.
- Never touch the peptide microarray slide surface.
- Always wear powder-free laboratory gloves when handling peptide microarray slides.
- Hold peptide microarray slides at the end, which carries the engraved data label. This label provides a unique identification of the specific microarray.
- Take care when dispensing solutions onto the slide surface. Make sure not to touch the surface with pipette-tips or dispensers.
- Inappropriate chemicals may destroy the chemical bonding of the peptides to the glass surface. Never use chemicals with corrosive activity.
- Usage of strong alkaline or acidic solutions should be avoided.
- Avoid dust or other particles during each step of the experiment. Dust, particles and resulting scratches will cause artifacts during the final signal readout.
- Filter all solutions for the washing steps through 0.2 µm particle filters before use.

READ THE ENTIRE PROTOCOL BEFORE STARTING YOUR EXPERIMENTS! CAREFULLY NOTE THE HANDLING AND STORAGE CONDITIONS OF JPT's PEPTIDE MICROARRAYS.
PLEASE CONTACT JPT PEPTIDE TECHNOLOGIES´ CUSTOMER SUPPORT FOR ASSISTANCE IF NECESSARY.

4 General Considerations

4.1 Experimental Basics

JPT Peptide Technologies' Multiwell 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 peptide to avoid false negatives caused by sterical hindrance. For technical reasons all peptides contain a C-terminal glycine.



JPT's 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 an analyte, a fluorescently labelled detection molecule is used for signal readout.

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

4.2 Assay Principle

The most common application of JPT´s peptide microarrays is the epitope mapping procedure (Figure 1). A schematic view of the assay principle is shown in Figure 2.

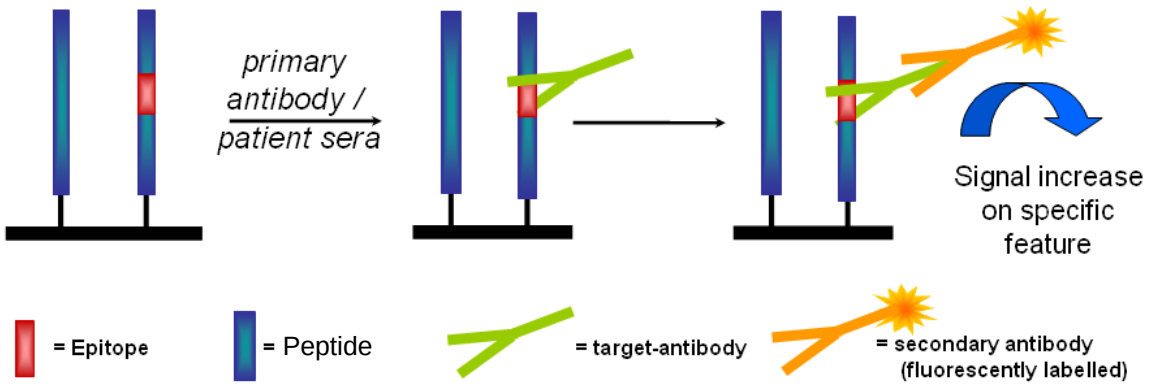


Figure 2: Peptide microarray assay principle.

The peptide microarray is incubated using a primary antibody or patient sample – e.g. serum, plasma or saliva – for a defined time. This enables the formation of stable peptide-antibody interactions. Subsequent to this incubation, the fluorescently labelled secondary antibody is applied. Bound to the peptide-bound primary antibodies, the fluorescence label of the secondary antibody enables readout of antibody interaction by microarray scanning systems. Each spot that shows an interaction with the primary antibody will gain signal in the resulting scan-image.

	It is crucial to perform control incubations in order to distinguish between real signals and false positives. To avoid false positive signals induced by peptide-secondary antibody interaction, JPT recommends performing regular control incubations using secondary antibodies only. In addition, JPT recommends performing control incubations applying unrelated primary antibodies to check for false positive signals induced by interaction of peptide with Fc-fragment of the primary antibody.

	For seroscreening application JPT recommends checking the secondary antibody for selectivity and specificity. Signals induced by cross-reactivity of secondary antibodies directed against IgG towards IgM or IgE may result in false positive results.

4.3 Multiwell Peptide Microarray Layout

Please refer to the .gal-file provided together with data files 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 JPT's GalViewer-software (part of data files sent by customer support). Since .gal-files are tab-separated text files, they can also be processed with software modules such as Microsoft Editor (Notepad) or Microsoft Excel. 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. A schematic layout of the Multiwell peptide microarray variants is shown in Figure 3.

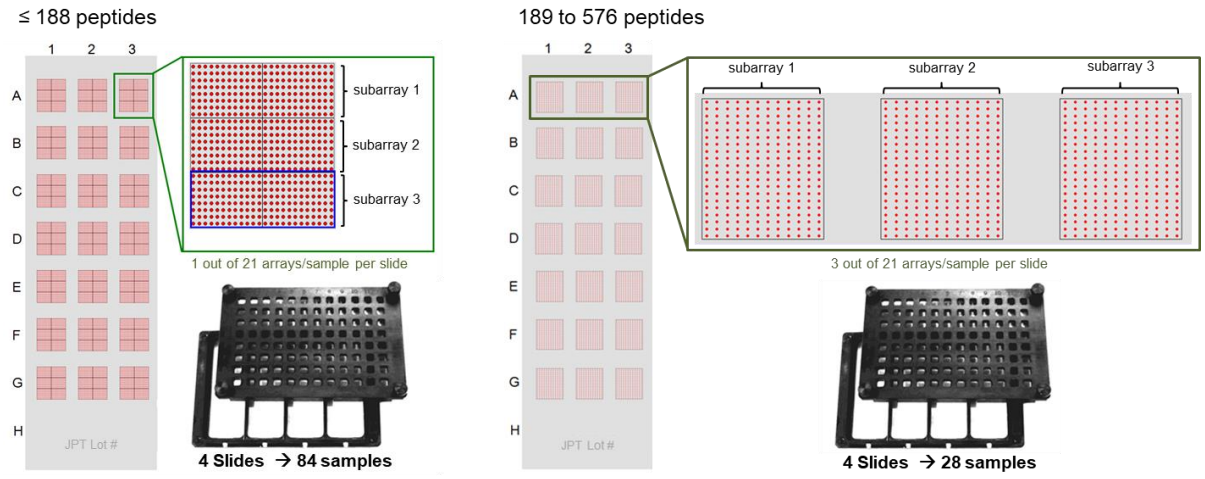


Figure 3: Schematic layout of Multiwell peptide microarray variants.

On Multiwell peptide microarrays, all peptides are printed in 21 identical mini-arrays on each slide. Depending on the peptide number each of these mini-arrays contain either three identical subarrays (≤ 188 peptides) or only one set of peptides (189 to a maximum of 576 peptides).

Since JPT recommends the incubation of each sample on three individual sets of the peptide library, either 21 samples (Figure 3 left: 21 mini-arrays with 3 subarrays

each) or 7 samples (Figure 3 right: 3 mini-arrays per sample) can be processed in parallel. This enables highly efficient intra-chip-reproducibility tests using scatter plots or correlation functions. The slide surfaces are delivered in a pre-treated form, minimizing unspecific binding of the target protein. Usually, therefore no blocking step is needed.

Using the 96-well microarray incubation chamber, depending on the peptide number 84 or 28 samples can be incubated on 4 slides simultaneously (Figure 3). The format of the 96-well microarray incubation chamber corresponds to a standard 96-well plate format and allows therefore the usage of standard ELISA equipment – incubators and washers. Due to the engraved label indicating the unique microarray-lot-number, row H is not utilizable as shown in Figure 3.

5 Experimental Protocols

Note: The following procedure is given as a guideline only. The optimal experimental conditions will vary depending on the investigated sample and instruments used and can, therefore, not be predetermined. The optimal experimental conditions must be established by the user. No warranty or guarantee of performance using this procedure with a target antibody or serum can be made or is implied.

The Multiwell peptide microarray is designed as a ready-to-use product to identify epitopes, peptide binders or immunodominant regions in antigens. Ordinary, there is no need to perform blocking steps on the slide surface prior to incubation with the target sample. Please refer to the .gal-files for identity and location of the spots on the peptide microarray surface. The side of the slide displaying the peptides is marked with the engraved lot number.

5.1 Additional Materials Required

1. **Analyte:**
   a. **Primary antibody**

      JPT recommends a final concentration of about 1 µg/ml

   b. **Proteins / enzymes**

      For analysis of e.g. protein binding components, JPT recommends a final concentration of 0.1 µg/ml or above, depending on the reactivity of the analyzed sample

   c. **Blood sera or plasma solution**

      Final sample dilution of 1:100 to 1:500 in blocking buffer

2. **Secondary antibody**

   Fluorescently labeled 2nd antibody (Note: JPT recommends DyLight 649 or related far-red-fluorescing dyes and a final conc. of about 1 µg/ml. Blue and green dyes are not recommended due to background issues.)

3. **Blocking buffer**

   For sample dilution (e.g. 3% BSA in 1x TBS-Buffer + 0.1% Tween20 (TBS-T))

4. **Washing buffer**

   1x TBS-Buffer + 0.1% Tween20 (TBS-T)

5. **De-ionized water**

   For final washing steps of the microarrays

6. **Silicone Grease**

   For the gasket of the 96-well microarray incubation chamber e.g. Bayer Silicon Grease Baysilone (medium viscosity)

5.2 Additional Hardware and Software

1. ***Tweezers (optional)***

   For handling of peptide microarrays

2. **96-Well Microarray Incubation Chamber**

   Required for incubation of micoarrays with multiple samples

3. ***4-Well Dish (optional)***

   JPT recommends performing all incubation steps using the 96-well microarray incubation chamber. Alternatively, 4-well dish, microscope slide staining dish or 50 ml-falcon tubes can be used for secondary antibody incubation.

4. ***ELISA/Mircoplate Washer (optional)***

   Alternatively, the 96-well microarray incubation chamber may be washed manually like a conventional ELISA plate.

5. **Orbital Shaker**

   For the incubation/shaking of the 96-well microarray incubation chamber

6. ***Rocking Platform (optional)***

   For the incubation/shaking of the 4-well dish

7. ***Slide Centrifuge (optional)***

   Alternatively, the slides may by dried by a gentle stream of nitrogen.

8. **Fluorescence Scanner/Imager**

   Capable of excitation of appropriate fluorophore moiety and with a resolution of at least 10 µm per pixel

9. **Analysis Software**

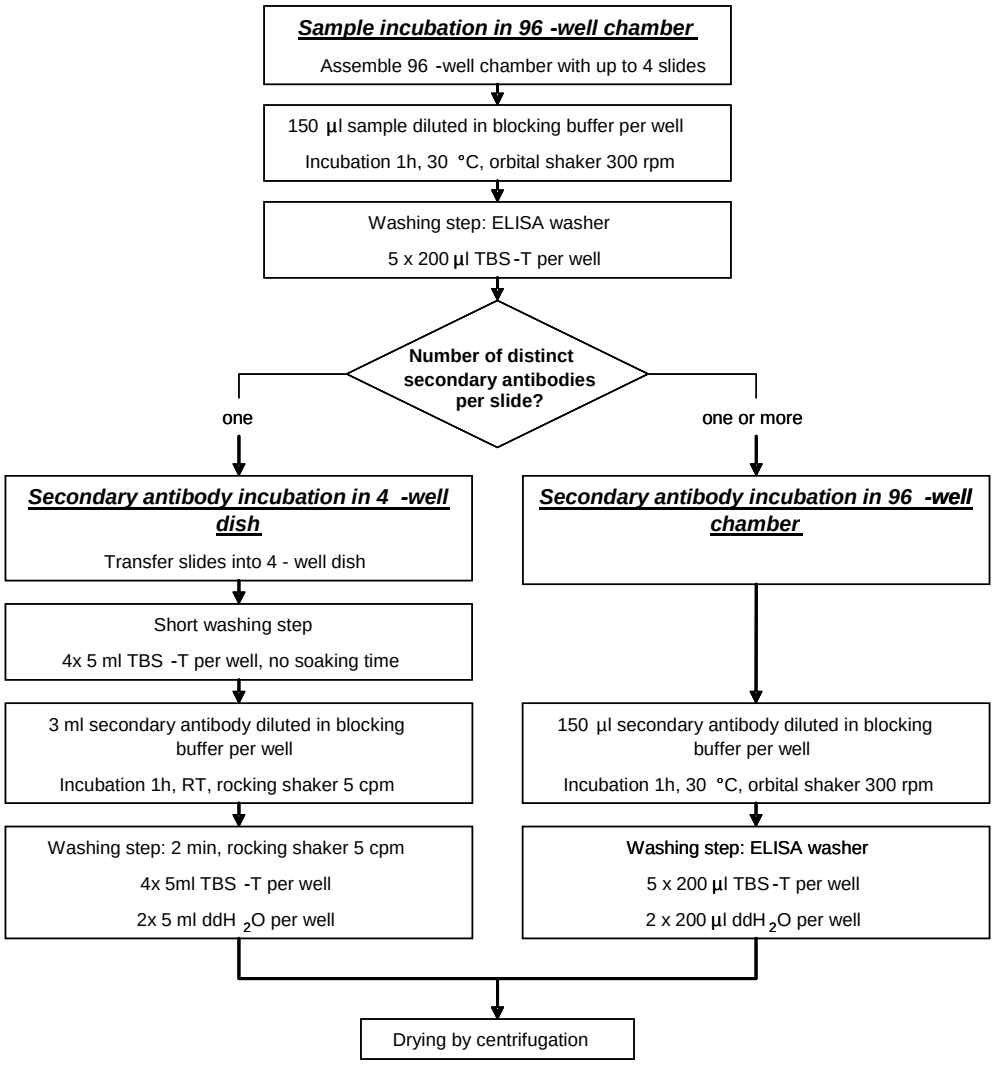
   Allowing quantification of the image and the assignment of signal intensities to individual peptides using the provided gal-file

5.3 Incubation Procedure

5.3.1 Microarray Processing

All Multiwell 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. All Multiwell peptide microarrays produced by JPT are adjusted to fit into the 96-well microarray incubation chamber allowing parallel processing of 84 or 28 samples using four slides depending on the peptide number (Figure 3).

5.3.2 Workflow of Multiwell Peptide Microarray Incubation



5.3.3 Assay Using 96-Well Chamber and 4-Well Dish (optional)

Assemble 96-well chamber with up to 4 slides. If less than 4 peptide carrying slides are used, please use enclosed dummy slides for vacant positions. Only if all 4 slide positions are occupied the chamber will seal the compartments properly.

1) Sample incubation
    - 150 µl sample diluted in blocking buffer per well
    - 1 h, 30°C, shaking 300 rpm (orbital)
2) Washing step using ELISA washer
    - 5x 200 µl TBS-T per well

Transfer slides into 4-well dish

3) Short washing step
    - 4x 5 ml TBS-T per well, no soaking time
4) Secondary antibody incubation
    - 3 ml secondary antibody diluted in blocking buffer per well
    - 1 h, RT, shaking 5 cpm (rocking)
5) Washing step
    - 4x 5 ml TBS-T, 2 min soaking, shaking 5 cpm (rocking)
    - 2x 5 ml ddH_2O, 2 min soaking, shaking 5 cpm (rocking)
        - Do not remove the water after the last washing step: leave the slides in water until drying.
6) Drying by centrifugation

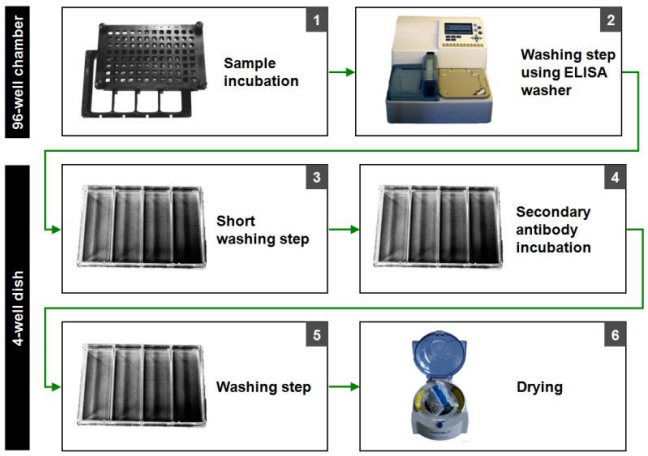


Figure 4: Multiwell array incubation procedure using 96-well chamber and 4-well dish.

5.3.4 Assay Using 96-well chamber only (recommended)

Assemble 96-well chamber with 4 slides

1) Sample incubation
    - 150 µl sample diluted in blocking buffer per well
    - 1 h, 30 °C, shaking 300 rpm (orbital)
2) Washing step using ELISA washer
    - 5x 200 µl TBS-T per well
3) Secondary antibody incubation
    - 150 µl secondary antibody diluted in blocking buffer per well
    - 1 h, 30 °C, shaking 300 rpm (orbital)
4) Washing step using ELISA washer
    - 5x 200 µl TBS-T per well
    - 2x 200 µl ddH_2O per well
5) Drying by centrifugation

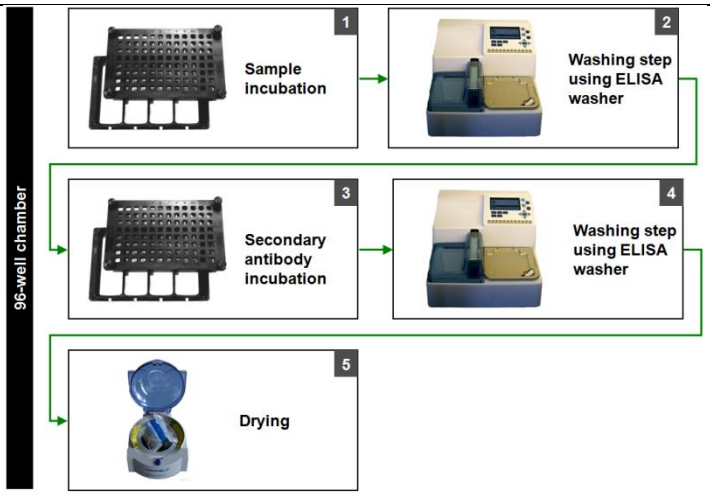
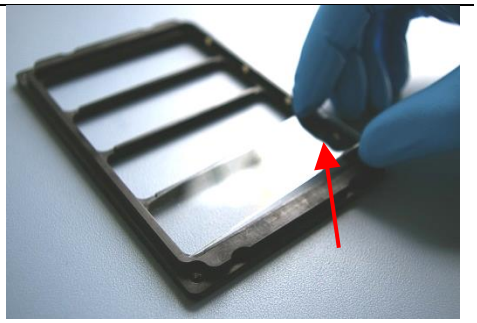


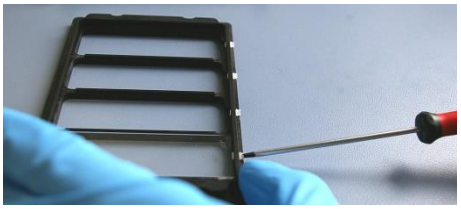
Figure 5: Multiwell array incubation procedure using 96-well chamber only.

5.3.5 Assembly of 96-Well Chamber

1) Insert the slides into the lower part of the chamber and fix them (one by one).

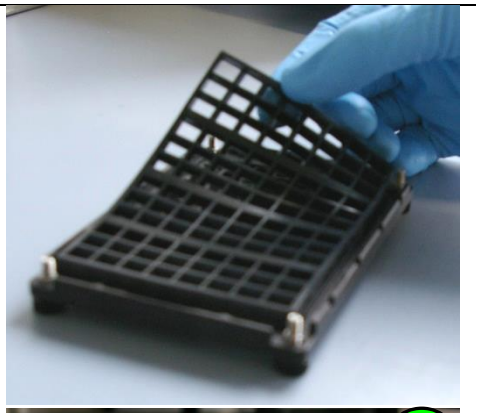
Orientation: JPT lot number must be readable and placed in row H.

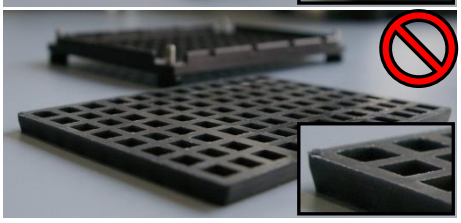




2) Insert the gasket into the upper part of the chamber.

Orientation: Thin side up.

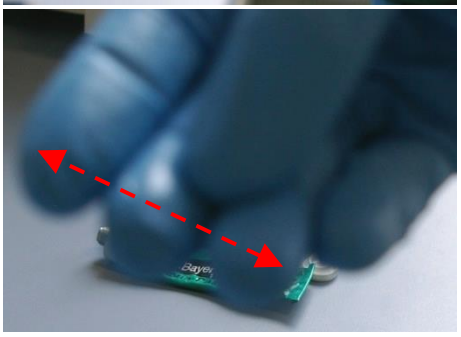


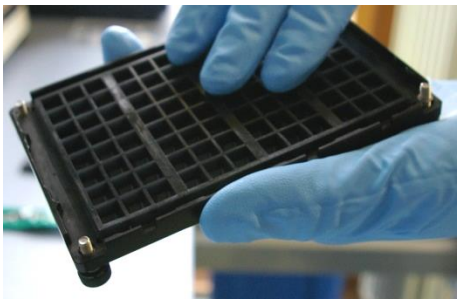


3) Make sure the gasket is sitting properly.

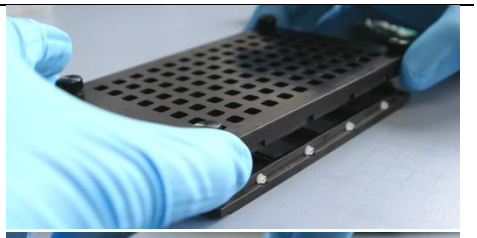
4) Put a **very thin** layer of silicone grease on the gasket.

- Take a tiny amount of silicone grease (less than on the picture).
- Spread it between your fingers to create a thin layer.
- Carefully, apply the silicone grease on the gasket to create a uniform film on all surfaces touching the microarrays.





5) Close and fix the chamber using the four screws.



5.3.6 Remarks

Washing:

- The settings of ELISA (microplate) washer should be adjusted in advance using standard glass slides. The washing head must not touch the slide surface.
- Alternatively, the 96-well chamber may be washed manually like a conventional ELISA plate.

Drying:

- Alternatively to using a slide centrifuge, the slides may by dried by a gentle stream of nitrogen.

6 Notes / Troubleshooting

Problem	Cause	Solution
Artifacts	• Dust particles and resulting scratches	• Avoid dust or other particles during each step of the experiment • Use filtered buffers and solutions only • When using ELISA washer, increase the height of the washing head. • Reduce the amount of silicone grease used.
High background	• Nature of the sample	• Direct fluorescently labelled proteins tend to induce back-ground signals via unspecific binding to the slide surface. Changing of buffer conditions in the incubation step can reduce background signals very efficiently • Additional washing steps can reduce non-specific binding • Variation of blocking buffers (usually, initial blocking steps are not recommended by JPT)
	• Sample / 2nd antibody concentration	• Increased concentrations of sample / 2nd antibody may yield high background signals caused by unspecific binding to the slide surface
	• Insufficient washing	• Adjustment of washing conditions
	• Contaminated wash buffer	• All buffers and solutions should be prepared freshly every day
Saturated peptide spots	• Concentration of the 2nd antibody	• Higher dilution rates of the 2nd antibody
	• Scanning conditions	• Adjustment of scanning parameters

Unspecific signals	• Nature of the sample	• Variation of blocking buffers
	• Insufficient washing	• Adjustment of washing conditions
	• Specificity of the 2nd antibody	• Control incubations using labelled 2nd antibody alone should be performed in parallel to the actual experiment to ensure that found signals are not caused by non-specific binding of the 2nd antibody to the immobilized peptides
Little or no signals	• Incubation time	• Warranty of sufficient incubation time
	• Bleaching effects	• During the incubation step with fluorescently labelled 2nd antibody, protect the slides from light! • After application of secondary antibody keep slides in an ozone-free environment
	• Scanning conditions	• Adjustment of scanning parameters

7 Related Products

For further information visit our homepage (www.jpt.com) or contact our customer support team (peptide@jpt.com).

- PepStar™: customized peptide microarrays displaying individually synthesized peptides in triplicates on one microarray
- PepSpots™: customized peptide arrays on cellulose membranes
- Peptide ELISA: customized peptide coated microtiter plates
- Ready to use RepliTope™ Microarray Kit containing all components and materials for your successful experiment (e.g. one-time incubation chamber, labelled antibody and buffers).