Protocol

Acetylome Peptide Microarrays

Ready-to-use peptide microarrays

Revision 1.2

Contact us:	
InfoLine:	+49-30-6392-7878
Order per fax:	+49-30-6392-7888
or e-mail:	peptide@jpt.com
www:	**www.jpt.com**

JPT Peptide Technologies GmbH
Volmerstrasse 5
12489 Berlin
GERMANY

Table of contents

1 Introduction

In the mammalian proteome, thousands of acetylation sites are known. Acetylation and deacetylation of proteins play an important role in regulation of gene expression, metabolism and can be linked to ageing processes as well as different types of diseases. This type of posttranslational modification (PTM) is accomplished by a variety of enzymes, related to the classes of acetyltransferases and deacetylases. The characterization of these classes of enzymes is essential for understanding their biological function.

One of the most efficient ways to investigate acetylation and deacetylation events is incubation of a collection of peptides representing known mammalian acetylation sites displayed on peptide microarrays with targets of interest. JPT Peptide Technologies' *Acetylome Microarrays* are peptide microarrays designed for rapid screening of e.g. acetyl-specific antibodies or acetyl group transferring enzymes. The *Acetylome Peptide Microarrays* consist of 13meric peptides comprising six residues N- and C-terminal from the acetylation sites. The peptides were synthesized in acetylated and nonacetylated form. *Acetylome Peptide Microarrays* were generated by chemoselective immobilization of these peptides *via* the N-terminus onto modified glass slides. The microarray can be used for broad and efficient mapping of antibody specificity or activity and selectivity of enzymes.

Upon incubation with your protein, antibody or sample of interest, the binding event can be detected by reading fluorescence intensity. For this purpose, either directly labeled proteins or labeled secondary antibodies can be used.

2 List of components

Component	Quantity
Acetylome Peptide Microarray	Glass slide displaying peptides in three identical sub arrays.
Blank slides engraved with *“Blank”*	One blank slide per Acetylome Peptide Microarray.
Spacers	Vials containing 20 spacers each.
Product Documentation	Relevant data files (peptide list as table, protocols as pdf-file and sequence info as gal-file).

3 Storage and handling

3.1 Storage of peptide microarray slides

- Optimal storage conditions for peptide microarray slides are in a cool (approx. 4°C / 39°F), dark 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.

3.2 Handling of peptide microarray slides

- Always handle the peptide microarrays with care.
- Never touch the peptide microarray slide surface.
- Never wipe or touch the surface of the peptide microarray slide with a cloth.
- Always wear 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 array.
- 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. Avoid usage of strong alkaline or acidic solutions.
- Avoid dust or other particles during each step of the experiment. Dust, particles and resulting scratches will cause artifacts during the final signal readout.
- Preferably 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´ TECHNICAL SERVICES FOR ASSISTANCE IF NECESSARY.

4 General considerations

4.1 Experimental basics

JPT Peptide Technologies' *Acetylome Peptide Microarrays* comprises peptides derived from 5599 human acetylation sites in 2644 proteins. The deposited peptides are chemoselectively and covalently linked to the glass surface via the C-terminus. An optimized hydrophilic linker is inserted between the glass surface and the antigen derived peptide to avoid false negatives caused by sterical hindrance.
JPT's peptide microarray slides are delivered in a pre-treated form minimizing unspecific binding of your target antibody or protein to the slide surface. Therefore, no blocking step is needed.

4.2 Peptide microarray layout

Please refer to the gal-file provided with the product documentation for the identity and location of the spots on the microarray surface. The side of the slide with the engraved label is the surface displaying the 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 1. The peptides are printed in three identical sub arrays on the microarray. This enables efficient intra-chip reproducibility tests. Each subarray is printed in individual blocks.

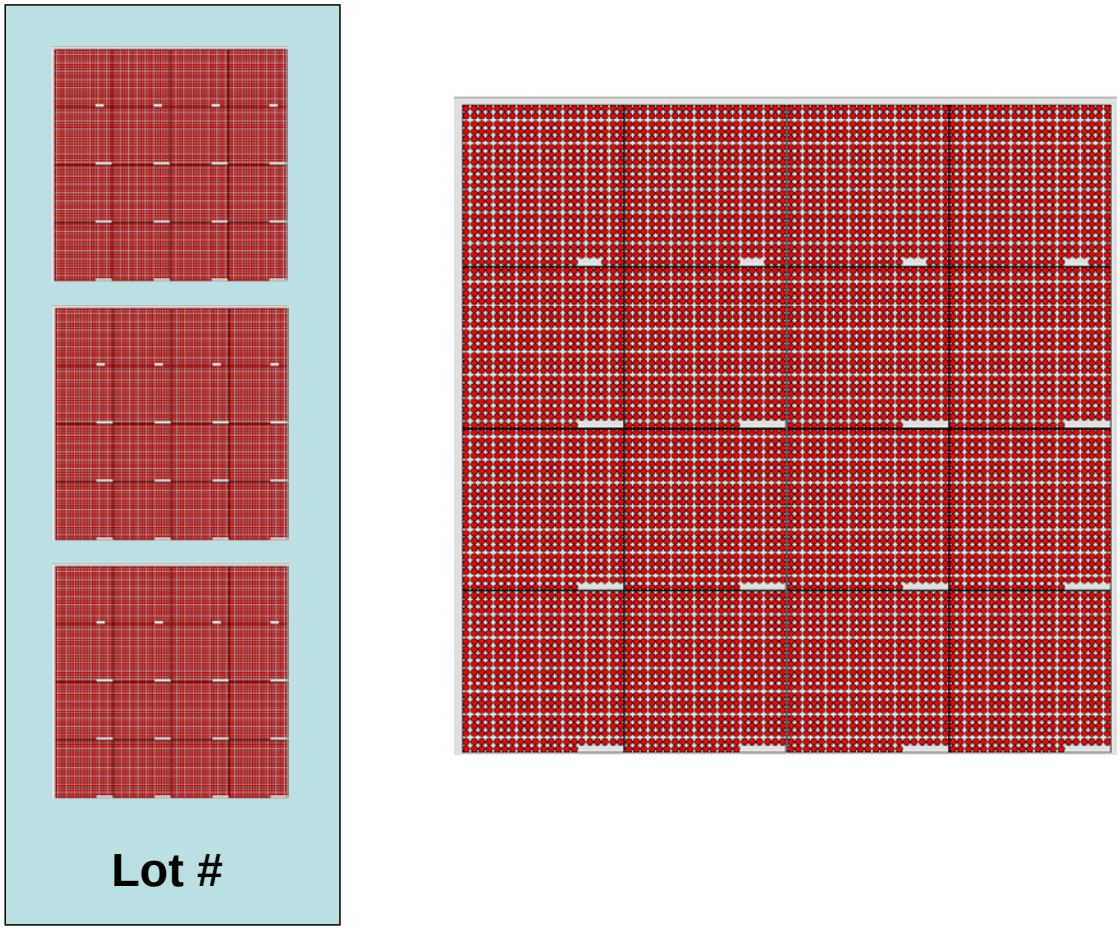


Figure 1: Exemplary view for a microarray slide with three 18 x 18 mm sub arrays (left) and a subarray consisting of 16 individual blocks (right).

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 your target antibody or serum can be made or is implied.

The *Acetylome Peptide Microarray* is designed as a ready-to-use product. 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 blood sera or plasma, JPT recommends to include an additional blocking step prior to incubation with patient sample.

Please refer to the gal-files provided with the product documentation for the 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 and solutions required

Component	Recommendations / Remarks
Primary antibody:	For high selectivity of acetyl-specific antibodies JPT recommends an optimized mix of two different antibodies: Acetylated-Lysine antibody (Cell Signaling Technology, #9441) PLUS Anti-Acetyl-Lysine antibody (Chemicon (Millipore), #3879) with a final concentration of 1:5000.
Proteins / enzymes:	For analysis of e.g. Lysin-modifying enzymes, JPT recommends a final concentration of 10 µg/ml or above.
Blood sera or plasma solution:	Final sample dilution of 1:100 to 1:500 in blocking reagent or assay buffer.
Secondary antibody:	Fluorescently labeled secondary antibody. JPT recommends Cy5 or related dyes and a final conc. of about 1 µg/mL. Blue and green dyes are not recommended due to background issues.
Tris buffered saline:	1x TBS-Buffer + 0.1% Tween20 (TBS-T)
De-ionized water:	For final washing steps of the microarrays.

5.2 Additional hardware and software

Component	Recommendations / Remarks
Automated incubation/ hybridization station	Tecan Hybridization Station HS4X00 *Note: Alternatively a microarray incubation sandwich can be used. Please refer to point 5.3.3 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 µm.
Software	Allowing quantification of the image and the assignment of signal intensities to individual peptides using the enclosed gal-file.

5.3 General principles for incubation

5.3.1 Enzymatic reactions using microarray-chip-sandwich

I.	**INCUBATION**	***WITH ACETYLTRANSFERASE OR DEACETYLASE CONTAINING SAMPLE*** Conditions can vary! They strongly depend on the nature of the enzyme! *Note: Final assay volume of the chip sandwich ~ 300 µl!*	
II.	**DISASSEMBLING**	in TBS-T	
III.	**WASHING**	with TBS-T	5x 3-4 min
		with de-ionized H_2O	5x 3-4 min

5.3.2 Detection of acetylation and deacetylation events

I.	**INCUBATION**	***WITH PRIMARY AC-LYS SPECIFIC ANTIBODY***
		@30°C (86°F) 2 hrs
		Note: Final assay volume of the chip sandwich ~ 300 µl !
II.	**DISASSEMBLING**	in TBS-T
III.	**WASHING**	with TBS-T 5x 3-4 min
IV.	**INCUBATION**	***WITH SECONDARY ANTIBODY***
		@30°C (86°F) 30-45 min
V.	**WASHING**	with TBS-T 5x 3-4 min
		with de-ionized H_2O 5x 3-4 min
VI.	**SLIDE DRYING**	Using microarray centrifuge / by blowing a gentle stream of nitrogen on the microarray surface
VII.	**IMAGING**	Fluorescence scanning
		Note: Scanning resolution = 10 µm pixel size !
VIII.	**DATA ANALYSIS**	Determination of signal intensities of each peptide spot.
		Bioinformatic evaluation of data.

5.3.3 Microarray incubation using microarray-chip-sandwich

To create a simple incubation chamber, two slides, one displaying the peptides and another slide (*Blank*-slide) without peptides, are assembled according to Figure 2 in a sandwich like format. Alternatively, two peptide carrying slides can be assembled to form an incubation chamber allowing further quality control of the experiment. 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.

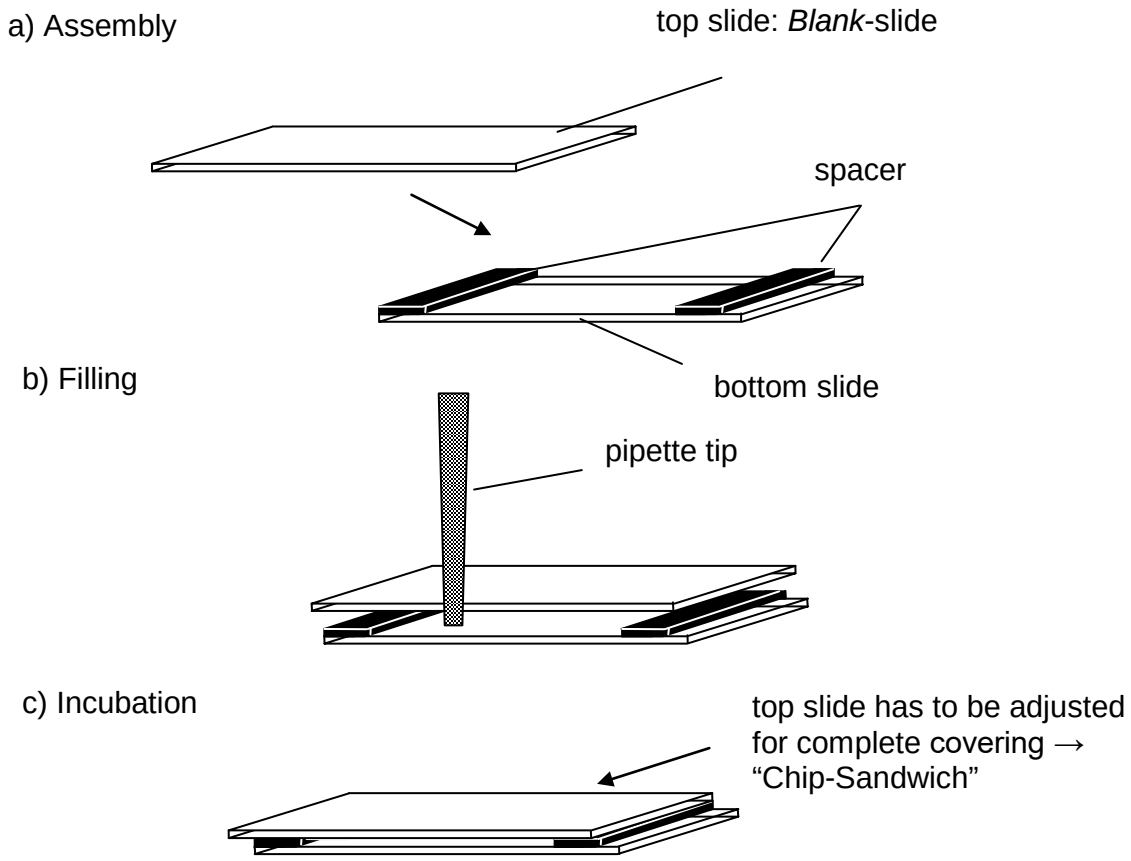
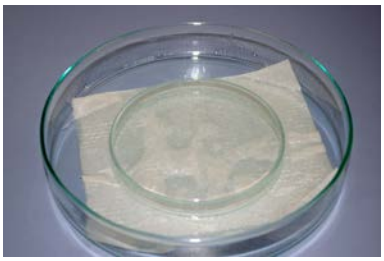


Figure 2: Microarray incubation using microarray-chip-sandwich.
a) For assembly of “Chip-Sandwich” two plastic spacers are placed between the peptide displaying microarray (bottom slide) and the *Blank*-slide or second peptide displaying microarray (top slide) resulting in a defined reaction chamber.
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 sample has to be applied in between the two slides. Therefore, the top slide is shifted about 1 mm in relation to the bottom 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.

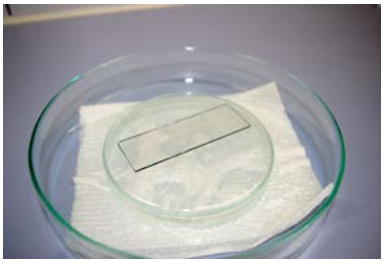
5.3.3.1 Preparation of the slide-environment for easy handling

IX. **WET-CHAMBER ASSEMBLY**



⇓

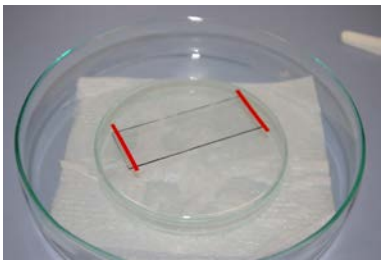
X. **PLACEMENT OF THE PEPTIDE MICROARRAY SLIDE SUPPORT PLATE FACING UPWARD**



Engraved label has to be readable from top.

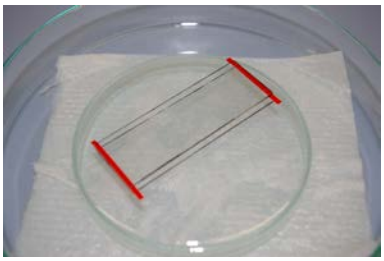
⇓

XI. **PLACEMENT OF ENCLOSED SPACERS ON BOTH ENDS OF THE MICROARRAY**



⇓

XII. ASSEMBLING OF THE MICROARRAY SANDWICH



See also 5.3.3. If two peptide microarrays are used make sure that peptide displaying sides are facing each other.

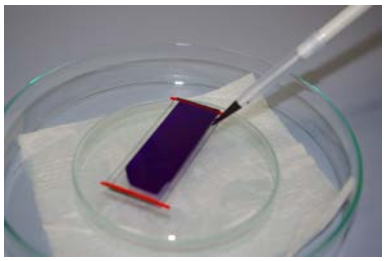
⇓

XIII. PREPARATION OF FINAL ASSAY SOLUTION CONTAINING TARGET ANTIBODY/ANALYTE

Approx. 300 µL if enclosed spacers are used.

⇓

XIV. PIPETTING OF THE COMPLETE VOLUME INTO MICROARRAY CHIP SANDWICH

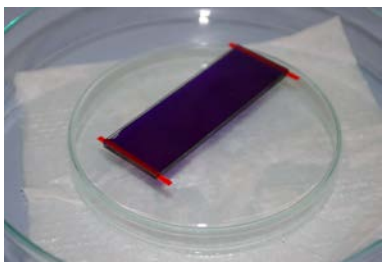


Capillary forces will suck the solution in between the two slides. Avoid air bubbles within the sandwich.

	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 artifacts!

⇓

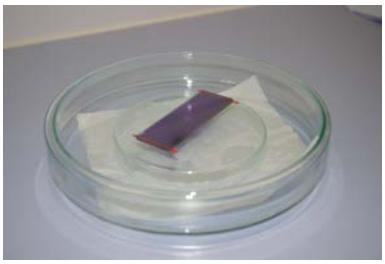
XV. ADJUSTMENT OF THE PEPTIDE MICROARRAY SANDWICH



As described in point 5.3.3 and Figure 2.

⇓

XVI. **CLOSING OF THE PETRI-DISH WITH A MATCHING COVER TO CREATE AN INCUBATION CHAMBER.**



5.3.4 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.

	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 Hybridization systems.

	It is strongly recommended to perform all enzymatic reactions in the described peptide microarray-chip-sandwich format! (*Please refer to point 5.3.3*)!

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

6 Notes / Troubleshooting

	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 to improved signal-to-noise ratio.

Comments and Suggestions

Additional blocking step	• Is not recommended by JPT. • In case of incubations with blood sera or plasma, JPT recommends to include an additional blocking step prior to incubation with patient sample.
Artifacts	• Avoid dust or other particles during each step of the experiment. • Dust particles and resulting scratches will cause artifacts during the final signal readout.
High background signals	• Carefully adjust the final dilution of your labeled secondary antibody. • Microarray technology is very sensitive. Usage of secondary antibody in a higher dilution as proposed by the manufacturer might be necessary. • Generally, the antibody concentration of 1 µg/mL was found working well in most cases. Depending on the nature of the secondary antibody, increased concentrations may yield high background signals caused by unspecific binding to the slide surface. • If the signals within the peptide spots are saturated, higher dilution rates of antibody are recommended. • Direct fluorescently labeled proteins sometimes tend to

	induce background 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. • Do not allow the slides to dry at anytime before the final drying step. • Scanning parameters should be adapted to signal intensities of the features.
Unspecific signals	• Control incubations using labeled secondary 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 secondary antibody to the immobilized peptides.
Little or no signals after fluorescence readout	• Enzyme is inactive: Check of incubation conditions (buffer, cofactors, temperature and time). • Activity of purified enzymes is higher than distinction of signals that can be obtained from biologic samples. • Carefully adjust the final dilution of your labeled secondary antibody. • During the incubation step with fluorescently labeled secondary antibody, protect the slides from light! • Change the settings and parameters of your fluorescence scanner to improve sensitivity of scanning. • Avoid any fluorescent impurities/contaminations inside your assay or wash solutions.

7 Service

Contact our customer support (peptide@jpt.com) for full service.

- Incl. *Acetylome Peptide Arrays* and all reagents
- Assay optimization (optional)
- Performance of your acetylome analysis
- Detection
- Quantification
- Short evaluation, resulting in a list of specific substrates or optional a detailed analysis

8 Related products

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

- PepStar™: customized peptide microarrays
- PepSpots™: customized peptide arrays on cellulose membranes
- Histone Code Peptide Microarrays