MILLIPORE

Protocol

Protease Peptide Microarray – Random Library

Ready-to-use microarrays for protease profiling

Catalog # 30-058

upstate
now part of Millipore

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I. Introduction

Enzymatic cleavage of peptide bonds in proteins represents a key regulation event in biologically important processes like signal transduction and cell cycle regulation. To study these processes and the involved enzymes, the identification of detailed substrate information is critical. One of the most efficient ways to study protease activities and substrate specificity is incubating a collection of potential substrate peptides displayed on peptide microarrays with the protease of interest.

Milllipore's Upstate Protease Peptide Microarrays, using JPT technology, are a selection of fluorescence labeled peptides derived from cleavage sites for rapid screening of protein protease activity. These peptides contain one fluorescein moiety at their C-terminal end distal to the microarray surface. Upon incubation with your enzyme, the cleavage of any peptide bond between the glass surface and the fluorescein can be detected by reading the fluorescence intensity before and subsequent to incubation with the enzyme.

II. Product Components

Component	Quantity
Protease Peptide Microarrays	glass slides each displaying 3 x 384 peptides
Disposable incubation chamber	depending on number of microarrays
Data CD-ROM	1 containing two files (protocol as pdf-file and sequence info as ecxel-file)

Storage:

- 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 month when stored at 4°C (39°F).
- Do not freeze the microarray slides for prolonged storage.

Handling:

- Always handle the delicate microarray slides with care.
- Never touch the microarray slide surface.
- Always wear laboratory gloves when handling Protease Peptide Microarrays.
- Please hold microarray slides at the end, which carries the engraved data label (frosted area). This label provides for unique identification of the array.
- Please take care when dispensing solutions onto the microarray surface. Make sure not to touch the surface with pipette-tips or dispensers.
- Never whisk the surface of the 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 artefacts during the final signal readout.
- Please filter all solutions for the washing steps through 2 µm, preferably 0.4 µm particle filters before use.

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING YOUR EXPERIMENTS! CAREFULLY NOTE THE HANDLING AND STORAGE CONDITIONS OF THE PROTEASE PEPTIDE MICROARRAYS. PLEASE CONTACT UPSTATE TECHNICAL SERVICES FOR ASSISTANCE IF NECESSARY.

III. Product Description

Millipore's ready-to-screen Upstate Protease Peptide Microarrays are comprised of fluorescently labeled peptides derived from cleavage sites. An optimized hydrophilic linker moiety is inserted between both, the glass surface / cleavage site peptide and the cleavage site peptide / fluorescein moiety to avoid false negatives caused by sterical hindrance. The peptides are displayed in three identical subarrays resulting in four glass slides with 3 x 384 = 1152 immobilized peptides on each slide. The four included peptide microarrays, each displaying 384 peptides, comprise a total of 1,536 fluorescently labeled peptides containing 21,504 potential cleavage sites. Screening of the complete or partial library results in the identification of both peptidic substrates and potential *in vivo* substrates of the target protease. The microarray has to be imaged before incubation with the target protease. Subsequent to incubation with the enzyme, cleaved peptide bonds can be detected by imaging the microarray again. Cleaved peptides will result in a decrease of fluorescence signal intensity.

The data CD-ROM included with the microarrays contains all information needed for the detailed analysis of your data including peptide sequences as well as the Swiss-Prot accession numbers for the proteins containing these cleavage sites. In case the individual peptide sequence is also found in other organisms, all respective Swiss-Prot accession numbers are given.

IV. Additional Materials Required

- Protease of adequate activity (we recommend a final activity of at least 1 U per mL).
- Protease assay buffer.
- Double distilled water and methanol for washing of the slides.
- Fluorescence scanner/imager capable of excitation of fluorescein moiety and with a pixel size of at least 50 µm. Pixel sizes smaller than 50 µm will result in more accurate data points but are not neccessary.
- Software tool allowing the assignment of signal intensities to spots on the surface of the microarrays.

V. Assay Procedure

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 enzyme can be made or is implied.

The Protease Peptide Microarray is designed as a ready-to-use product to assay enzyme activity directly on the surface of the glass slides. There is no need to perform blocking steps on the surface prior to incubation with the target enzyme. However, if you would like to perform an additional blocking reaction, please ensure to only use protein-free solutions like PEGs (polyethyleneglycols, 0.5 mg/mL) or PVPs (polyvinylpyrrolidones, 0.1 mg/mL). This keeps your protease of interest from cleaving peptide bonds within the protein used for blocking! Protease reactions are performed in a final volume of about 330 µL per slide.

Scan the Protease Peptide Microarrays before incubation with the protease and store the image as a reference for final data analysis!
Please refer to the files on the enclosed CD-ROM and to the corresponding data sheets for the identity and location of the spots onto the microarray surface. The side of the slide with the engraved label represents the surface displaying the attached peptides.

We recommend the use of an incubation chamber for best results. In our hands, the disposable Gene-Frame™ from Abgene (# AB-0630, http://www.abgene.com) with a total assay volume of 300 µL worked best with our slides under a wide range of conditions.
The incubation may be performed with other incubation chambers as well without loss of quality. In this case please refer to the instructions of the manufacturer.

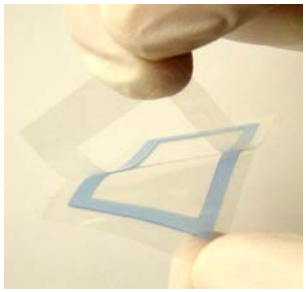
The following protocol describes the incubation of our Protease Peptide Microarrays using the disposable incubation chamber Gene-Frame™. It consists of two parts: a blue adhesive frame sandwiched between a thick and a thin polyester sheet, and a matching transparent coverslip.

When carrying out enzyme reactions in an incubation chamber, we recommend treatment the surface of the chamber prior to the enzyme reaction with a solution of 0.1mg/mL polyvinylpyrrolidone for at least 4 hours. If you are using the delivered Gene-Frame™ incubation chamber, it is sufficient to pre-treat the needed coverslip only.

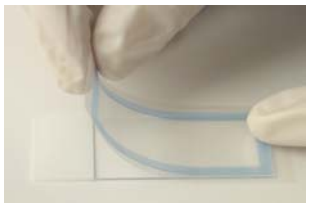
Attach chamber to peptide microarray

1. Ensure that the surface area of the Protease Peptide Microarray is dry and clean.

2. Each adhesive frame is sandwiched between a thin and a thick polyester sheet (while the thin sheet covers the adhesive frame completely, the thick sheet has the central part removed). Please remove the thick polyester sheet carefully ensuring that the blue frame stays on the thin polyester sheet.

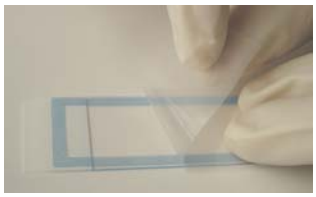


3. Carefully place the adhesive blue frame on the thin polyester sheet onto the microarray avoiding contact with the peptide displaying microarray surface.



4. Press down the blue frame firmly without trapping air under the adhesive.

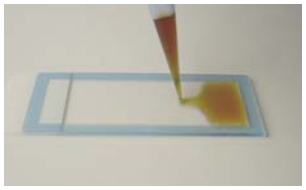
5. Remove the second, thin polyester sheet from the top of the adhesive blue frame.



Pipette protease solution into chamber

6. Prepare approximately 330 µL of final assay solution containing protease, buffer and additives needed for full protease activity like metal ions.

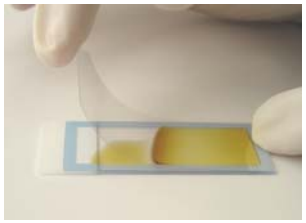
7. Pipette the complete volume starting at one end of the adhesive frame (For visualization only, the solution in the figure is in yellow!).



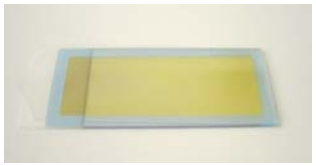
Make sure that microarray surface will not be touched by the pipette tip. Scratches and marks on the surface will cause artefacts during the fluorescence scanning.

Seal incubation chamber

8. Carefully place the pre-treated polyester coverslip over the adhesive frame. Start at the end where the assay solution was first pipetted on and slowly press down the coverslip over the blue frame.



9. The deposited solution will then spread across the whole microarray surface without trapping any air bubbles.



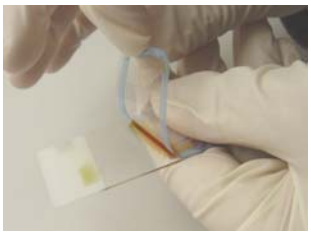
10. Press the cover onto the adhesive frame with a blunt instrument for approximately 10 seconds.

Incubate

11. Incubate the peptide microarray for appropriate time and temperature with the protease. Millipore recommends an incubation time of at least 1 hour at approximately 30-35°C (86-95°F).

Remove incubation chamber

12. To remove the incubation chamber after incubation hold the slide with one hand and gently pull back the tab of the coverslip along the length of the slide. Use the disposable incubation chamber for one experiment only.



Take care to remove the incubation chamber completely. Any remaining adhesive might disturb the following washing steps.

Washing

13. Wash the microarray slides 5 times for 5 min each with double distilled water followed by washings with methanol. Take care that the slide was washed using sufficient volumes of solution. Subsequent to washing dry glass slides using either a stream of nitrogen or using a slide centrifuge. Avoid drying of solvent droplets directly onto the slide surfaces.

Imaging of Protease Peptide Microarray

14. Perform fluorescence scans according to your scanner type. We recommend resolution with a pixel size of at least 50 µm.

Data analysis

15. Generate lists containing signal intensities of each spot before protease incubation and subsequent to protease incubation.
16. Calculate the mean value for the signal intensities of spots with identical peptides (three identical subarrays) for the image before protease incubation (A) and subsequent to protease incubation (B).
17. Calculate the ratio between B and A (B/A). All values should be smaller or < 1!
18. Arrange the results according to the B/A value. Start with smallest value. The smallest values indicate the spots displaying peptides cleaved most effectively by the protease.
19. Align identified top 25 substrates to extract consensus amino acids or sequence motifs.

VII. Notes

- 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.
- Proteases may have diverse optimal assay conditions (ionic strength, pH value, necessary additives like metal ions etc.). Therefore, a universal buffer system cannot be provided.
- Blocking with protein containing solutions like bovine serum albumin in tris buffer can cause a signal decrease of the fluorescently labeled peptides on the microarray by unspecific binding to the immobilized peptides impairing the final results. If you need such a blocking step (not recommended by Millipore!) please scan the microarray subsequent to this blocking step and use that image as a starting point for your analysis.

Warranty

These products are warranted to perform as described in their labeling and in MILLIPORE® literature when used in accordance with their instructions. THERE ARE NO WARRANTIES, WHICH EXTEND BEYOND THIS EXPRESSED WARRANTY AND MILLIPORE® DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. MILLIPORE®*'s* sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of MILLIPORE®, to repair or replace the products. In no event shall MILLIPORE® be liable for any proximate, incidental or consequential damages in connection with the products.

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