

Protocol

ProteaseSpots

Ready-to-use cellulose bound peptides for protease profiling

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1 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 way to study protease activities and substrate specificity is incubating a collection of potential substrate peptides bound onto cellulose discs with the protease of interest.

JPT Peptide Technologies' ProteaseSpots are customized fluorescence labelled peptides for rapid screening of protein protease activity. These peptides contain a fluorescent moiety at their N-terminal end distal to the cellulose surface. Upon incubation with your enzyme, the cleavage of any peptide bond between the cellulose and the fluorophore can be detected by reading the fluorescence intensity before and subsequent to incubation with the enzyme.

2 Product Components

Component	Quantity
ProteaseSpot peptides	delivered in 96-well plates (Greiner Bio One, #655101, flat bottom)
Dokumentation	microplate scheme, sequence list and protocol

3 Storage and Handling

- Optimal storage conditions for ProteaseSpot peptides are in a cool (approx. -20°C / -4°F) and dry environment.
- Always handle the 96-well plate with care.
- Always wear laboratory gloves when handling ProteaseSpot peptides.
- Remove the silicon cover very carefully due to possible electrostatic charge.

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING YOUR EXPERIMENTS! CAREFULLY NOTE THE HANDLING AND STORAGE CONDITIONS OF THE PROTEASESPOT PEPTIDES.

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

4 Product Description

JPT Peptide Technologies' ready-to-screen ProteaseSpot peptides are customized fluorescently labelled peptides. The peptides are bound onto cellulose discs in 96-well plates. Subsequent to incubation with the enzyme, cleaved peptide bonds can be detected by analysing the enzyme/cleaved peptide solution by pipetting the solution in a daughter plate. Cleaved peptides will result in an increase of fluorescence signal intensity in the daughter plate.

5 Additional Materials

- Protease of adequate activity
- Protease assay buffer.
- Methanol
- Fluorescence scanner/imager capable of excitation of fluorescence moiety

6 Assay Description

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.

6.1 Assay Principle

The protease assay is based on peptides synthesized on continuous cellulose membranes using the SPOT-synthesis technique. The peptides have a fluorescent moiety at the N-terminus and are punched out as small discs into 96-well microplates. A solution with protease activity is added and after different times aliquots are transferred into daughter plates. Proteolytic cleavage of the N-terminal part of the peptide is quantified in a fluorescence microtiter-plate reader.

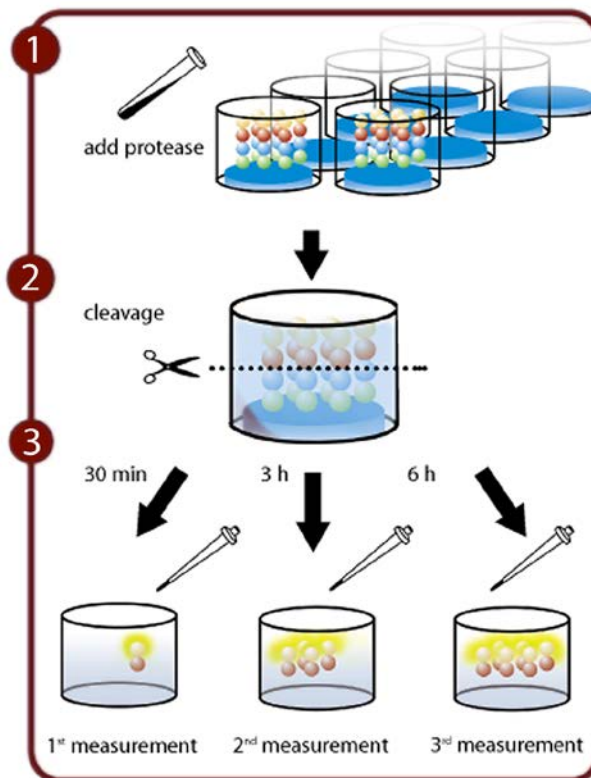


Figure 1: schematic demonstratation of assay principle

6.2 Assay Protocol

The spots are delivered in 96-well microplates and are numbered according to the enclosed sequence list and microplate scheme. Individual spots can only be identified by their position in the plate since the spots themselves do not have any numbering! If additional plates have to be used, one should strictly avoid high binding capacity plates for protein coating. In order to compare measurements of different cleavage times it is recommended to use only plates of the same type for the transfer step.

1. Rinse the spots once for 5 min with 200 μ l methanol. This helps to solubilize in particular hydrophobic peptides.
2. Rinse the spots four times for 10 min under gentle agitation with 200 μ l of the desired protease assay buffer which is best suited for the respective protease. Keep in mind that the best fluorescence yield is obtained in the range between pH 7-9. Remove the buffer completely. Excess buffer results in poor comparability between different spots. However, avoid drying out of the spots.
3. Add 200 μ l of the protease solution. Compared to soluble substrates, slightly more protease activity is needed to compensate the limited diffusion of substrates bound to a solid support.
4. Seal the plate with a self-adhesive plastic sheet to minimize evaporation. Perform the assay under the optimum temperature for the protease. The peptides are stable up to at least 50°C.
5. Mix the solution above the cellulose disc by pipetting in and out a few times prior to taking 50 μ l aliquots after 1 h, 12 h and 36 h. Transfer aliquots into free wells of the same or another plate.
 - Turning the Spots upside-down has no influence on the results.
 - Leave at least one well free between the spots and the aliquots, since very high fluorescence yields are usually observed with the spots and this signal can affect the values for the lower aliquot yields. Depending on the microtiter-plate reader, black or white plates may be required.
 - A small amount of the solution remains in the disk.

- Incubation times can be varied.
 - More but smaller aliquots can be taken. In this case buffer should be added to a final volume of 50 μ l to cover the disk completely.
 - If the optimum pH is far off 7-9, a better fluorescence signal can be obtained by adding concentrated buffer to the aliquots prior to measurement.
6. Quantify the cleavage with a fluorescence microplate reader. The surface tension of the solutions in the daughter plates can vary depending upon the concentration of the released N-terminal part, which can lead to different menisci in the wells. It is therefore important to measure the entire well and not only the center. Most readers have the option to select the area of the wells to be quantified.
7. Avoid extensive UV excitation in order to reduce bleaching effects.

7 Notes

- All pipetting steps have to be carried out very carefully since they directly influence the final measurement.
- Differences in the range of 20-25% can be observed between results obtained for spots with the same sequence. This is the sum of errors in synthesis, pipetting, evaporation, bleaching and measurement.
- Fluorescence scanning could be very sensitive depending on the scanner. Avoid any fluorescent impurities / contaminations inside your assay solution or wash solutions.
- 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.
- Some examples of commonly used fluorescence tags and their wavelenghts of excitation and emmision:

name	abbr.	excitation	emission
2-aminobenzoic acid	Abz	325 nm	420 nm
5(6)-carboxyfluorescein	5(6)-FAM	494 nm	518 nm
5(6)-tetramethylrhodamin	5(6)-TAMRA	544 nm	572 nm

8 Related Products

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

- Protease Substrate Set
- Caspase Substrate Set
- Protease peptide microarray
- Protease Profiling Service