MILLIPORE

# Protocol

# Caspase Substrate Set

## Ready-to-use Peptide Set for Protease Profiling

**Cat. No. 30-056**

**FOR RESEARCH USE ONLY**
**Not for use in diagnostic procedures.**

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## I. Introduction

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

The Millipore's Upstate Caspase Substrate Set, using JPT technology, is a selection of 4 x 88 peptides derived from cleavage sites for rapid screening of caspase activity. These peptide derivatives contain the cleavage site sequences from P4- to P4´-position, which is flanked by DABCYL and Glu(EDANS)-amide moieties at the N-terminus and C-terminus, respectively. Upon incubation with your protease, cleavage of any peptide bond between the fluorophore and the quencher moiety can be detected using microtiter plate fluorescence readers.

## II. Product Components

| Component | Quantity | Format |
|---|---|---|
| Caspase Substrate Set | 1 | 384 well microtiter plate |
| Data CD-ROM | 1 | Microsoft Excel file |

**Storage:** Caspase Substrate Set microtiter plates should be stored at -20°C. All other components may be stored at room temperature.

**PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING YOUR EXPERIMENTS! CAREFULLY NOTE THE HANDLING AND STORAGE CONDITIONS OF THE PROTEASE SUBSTRATE SET.**

## III. Product Description

Millipore's ready-to-screen Upstate Caspase Substrate Set is comprised of a total of 4 x 88 peptides derived from cleavage sites described in the scientific literature. The Caspase Substrate Set comes in a 384-well microtiter plate (75 pmol per well). In addition, several empty wells are available for positive and negative controls (columns 1 and 13). Subsequent to incubation with the target caspase, evolving fluorescence can be detected using standard microtiter plate readers. The sensitivity of the assay is sufficient to detect low nanogram levels of protease. The peptide derivatives are purified by HPLC (>95% at 220 nm) and freeze-dried into the wells from a DMSO stock solution. There are no additional buffer salts inside the wells. The peptide derivatives are not immobilized onto the walls of the wells resulting in a homogeneous assay after dissolving of the peptides in assay buffer.

The data CD-ROM provided with the set contains all information needed for the detailed analysis of your data including peptide sequences and Swiss-Prot accession numbers for the proteins containing these cleavage sites. If the individual human peptide sequence could be found in other organisms, too, all appropriate Swiss-Prot accession numbers are given.

## IV. Additional Materials Required

- Protease of adequate activity (we recommend a final activity of 0.1 U per well)
- Protease assay buffer
- Microtiter plate reader capable of measuring fluorescence at 490 nm to at least 3-decimal accuracy. Wavelengths in the range of 450-520 nm may be used and will result in similar sensitivities. The excitation wavelength should be in the range of 340-360 nm. Excitation outside that range is possible but will reduce the total fluorescence intensity.

## V. Assay Procedure

The following procedure is recommended for the Caspase Substrate Set assay using a fluorescence reader which excites and reads fluorescence from the top of the plate. If your reader excites from the bottom and reads from the top or vice versa please contact JPT for Protease Substrate Sets delivered in appropriate microtiter plates. Please note that you will need to optimize buffer and reaction conditions for your specific target protease.

**Note: The following procedure is intended as a guideline only. The optimal experimental conditions will vary depending on the investigated parameters, and must be determined by the individual user. No warranty or guarantee of performance using that procedure is made or implied.**

The Caspase Substrate Set is designed for assaying the enzyme activity directly in the 384-well microtiter plate. Protease reactions are performed in a final volume of 15 µL resulting in a final peptide concentration of 5 µM in each well. **Before starting the experiment make sure that your enzyme preparation, water and buffer components do not contain contaminants or components which may interfere with the fluorescence signal.**

1. Let the Caspase Substrate Set adjust to room temperature.

2. Carefully remove the foil covering the microtiter pate.

3. Add 10 µL of assay buffer (without enzyme) to each well and allow the peptide derivatives to dissolve for 3 hours at room temperature.

4. Measure fluorescence at about 490 nm (excitation in the range of 340-360 nm). Store these data as a starting set and use the lowest value of the wells filled with peptide solution as a blank.

5. Transfer an adequate volume of assay buffer including the protease (we recommend 5 µL/well resulting in a total volume of 15 µL peptide solution per well) into each peptide-containing well. Add background controls to the wells A1-P1, A13-P13 (enzyme without substrate; assay buffer, components of the assay buffer like metal ions or reducing agents).

**Avoid bubbles during the addition of the enzyme solution. Bubbles in the wells of the plate will adversely affect the fluorescence readings. If bubbles are present, use a centrifuge to remove them.**

**Use at least the protease solution itself without any peptide as a control. We strongly recommend the use of all assay buffer components as single controls. Make sure that the final volume in all wells is similar.**

6. Incubate the microtiter plate with the protease solution for 20 min to 2 hours (45 min recommended) at 37°C. Please note that reaction conditions might need to be optimized for your protease regarding incubation temperatures and reaction time.

7. Measure fluorescence at about 490 nm (excitation in the range of 340-360 nm). We recommend to blank against the lowest fluorescence value of the wells filled with peptide solution from the measurement before the reaction was started (see point 4).

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## VI. Notes

- The fluorescent readout is a highly sensitive detection principle. Each fluorescent component in your final assay solution will greatly increase the background signal. Make sure that your assay components are free from small dust particles. Use filtrated solutions only (at least 0.45 µm pore size)!

- Proteases possess diverse optimal assay conditions. Therefore, a universal buffer system cannot be provided.

- Do not store the dissolved peptide solutions longer than one day! Depending on the peptide sequence, aqueous peptide solutions are unstable. Use freshly prepared peptide solutions only for the protease experiment.

- Each cleavage event between the fluorophore and the quencher moiety will result in evolving fluorescence. Therefore, this set can be used for the analysis of orphan proteases as well.

## 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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**Cat No.30-056**

January 2007

Revision A, 4003713