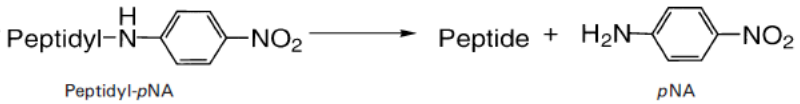
Assay Method Using Peptidyl-*p*NA Substrates

Principle

A protease with limited specificity hydrolyzes a peptidyl-*p*NA substrate, releasing *p*-nitroaniline (*p*NA) as follows:



The initial rate of increase in the *p*NA concentration can be monitored photometrically at 405 nm.

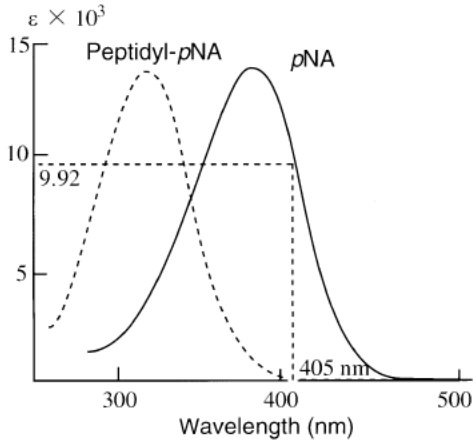


Fig. UV-Absorption Spectra

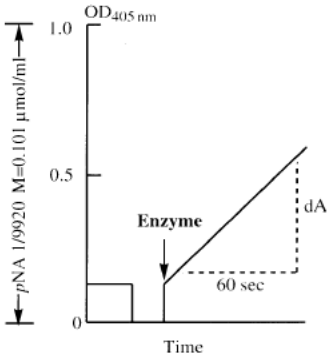
Reagents

1) Substrate stock solution: 10 mM (DMSO or distilled water)
2) Buffer
3) Enzyme solution

Procedure

Choose the proper conditions for the measurement, such as substrate concentration and sensitivity setting, depending on the purpose of the experiment and the instrument available. Described here is one of the recommended procedures for the photometric method (initial-rate method).

1) Set a spectrophotometer at λ= 405 nm at 25℃ ($\varepsilon_{405\ nm}$=9920)*
2) Pipette 2940 µl of buffer and 30 µl of substrate stock solution into the cuvette
3) Incubate in the spectrophotometer for 3-4 min (for temperature equilibration)
4) Add 30 µl of enzyme solution
5) Record the increase of the absorption intensity for 3-4 min
6) Calculate the amount of *p*NA released using the following equation



$$\text{Amount (µmol) of } p\text{NA released/min} = \frac{1\ \mu\text{mol} \times dA \times 3\ \text{ml}}{1\ \text{ml} \times 9.92 \times 1\ \text{min}} = 0.302 \times dA\ \mu\text{mol/min}$$

*R. Lottenberg, U. Christensen, C.M. Jackson, and P.L. Coleman, *In*, Proteolytic Enzymes Part C, *Methods in Enzymolology*, **Vol. 80**, (L. Lorand, ed.) Academic Press, New York, 1981, pp. 341-361.

Peptides International, Inc.
11621 Electron Drive • Louisville, KY 40299 • (502) 266-8787 • Fax (502) 267-1329

www.pepnet.com • peptides@pepnet.com