

Product Information

N-ACETYL-ILE-GLU-PRO-ASP - p-NITROANILIDE

Product Number **A 6470**
Storage Temperature -20°C

(Ac-IEPD-pNA)

Product Description

Appearance: White Powder
Formula Weight: 634.6
Molecular Formula $\text{C}_{28}\text{H}_{38}\text{N}_6\text{O}_{11}$
Purity: $\geq 97\%$ by HPLC.

Colormetric substrate for caspase 8 and granzyme B.

Preparation Instructions

Soluble in dimethyl sulfoxide (DMSO) to 20 mM.

Storage/Stability

Store tightly sealed and desiccated at -20°C . Allow powder to reach room temperature before opening vial. May be stored desiccated in solid form at room temperature for one year. Store DMSO solutions at -20°C for up to 6 months.

Procedure

Colormetric Enzyme Assay in 96-well ELISA Plate

- Buffer: 25 mM HEPES, pH 7.5, 0.1% CHAPS, 5 % (w/v) sucrose, 5 mM DTT, 2 mM EDTA. Use 17 Mohm deionized water.
- Substrate: 2 mM stock solution of Ac-IEPD-PNA in DMSO.
- Enzyme: Cell lysate or purified enzyme solution ($\sim 10\ \mu\text{g/ml}$ enzyme).
- p-Nitroaniline Standard: 200 μM free p-nitroaniline (Product Number N 2128) in DMSO.

1. Place 10 μl of the diluted cell lysate or purified enzyme solution in a well.
2. Add the 90 μl of Buffer to the well.
3. Start the reaction with the addition of 10 μl of the 2 mM substrate solution using a multichannel pipette.

4. Place in the ELISA reader and read at 5 minute intervals at 405 nm for t minutes (where t can be from 20 to 60 minutes or even longer for very dilute samples).
5. Calculate the net OD increase by subtracting the value at zero time from the value at the time interval measured.
6. Determine the amount of p-Nitroaniline (nmol) released during the reaction by comparing the net OD to the p-Nitroaniline (pNA) standard curve (see Table 1).

Table 1: p-Nitroaniline Standard Curve

nmol pNA per well	pNA std 200 μM μl per well	Buffer μl per well
0	0	100
1	5	95
2	10	90
5	25	75
10	50	50
15	75	25
20	100	0

Calculation

Calculate the enzyme activity as nmol pNA released per min per ml for the enzyme sample.

V = volume in ml of enzyme solution in the reaction
D = any dilution of original enzyme sample prior to addition to reaction.

t = reaction time in minutes

A_{nmol} = absorbance of 1 nmol in the microwell from the calibration curve

A_t = absorbance at time t min.

A_0 = absorbance at zero time

$$\text{Activity, nmol/min/ml} = \frac{(A_t - A_0) \times D}{(A_{\text{nmol}}) \times (t) \times V}$$