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ProductInformation

N-ACETYL-LEU-GLU-HIS-ASP-p-NITROANILIDE

Product Number **A 5970**
Storage Temperature –20 °C

(Ac-LEHD-pNA)

Product Description
Appearance: White Powder
Formula Weight: 674.7
Purity: >97 % by HPLC.

Colormetric substrate for caspase 9.

Preparation Instructions
Soluble in 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 % (v/v) sucrose, 5 mM DTT, 2 mM EDTA. Use 17 Mohm deionized water.
- Substrate: 2 mM stock solution of Ac-LEHD-pNA in DMSO.
- Enzyme: Cell lysate or purified enzyme solution (~10 µg/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 OD formed in the time interval measured minus the value at zero time.
6. Calculate the results in nmol using a p-Nitroaniline (pNA) calibration curve (see Table 1).

Table 1: p-Nitroaniline Calibration 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

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

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