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ProductInformation

N-Acetyl-VAL-GLU-ILE-ASP-p-Nitroanilide

Product Number **A 5220**
Storage Temperature –20 °C

(Ac-VEID-PNA)

Product Description
Formula Weight: 636.7

This product is a colorimetric substrate for caspase 6.

Preparation Instructions
Soluble in DMSO (2 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/DMF solutions at –20 °C for up to 6 months.

Procedure
Colorimetric Enzyme Assay in 96 well ELISA Plate

- Buffer: 25 mM HEPES, pH 7.5, 0.1% CHAPS, 5% (w/v) sucrose, 5 mM DTT, and 0.5 mM EDTA. Use 17 Mohm deionized water.
- Substrate: 2 mM stock solution of Ac-VEID-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 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 20-60 minutes or even longer for very dilute samples).
5. Measure the increase of A_{405} during the time interval and subtract the value at zero time.
6. Calculate the results in nmole using a p-nitroaniline calibration curve (see Table 1).

Table 1.
p-Nitroaniline Calibration Curve

nmole of pNA per well	200 µM pNA std 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 nmole of pNA released per minute 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_{nmole} = absorbance of 1 nmole in the microwell from the calibration curve
A_t = absorbance for time interval (t minutes)
A_0 = absorbance at zero time

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

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