



cell signaling solutions

Certificate of Analysis

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Caspase 3

(recombinant protein expressed in *E. coli*)

Catalog # 14-264

Lot # 26083

Product Description: Recombinant full length protein containing a C-terminal histidine tag; expressed in *E. coli*. Caspase-3 is a member of the ICE/CED3 family of cysteine proteases that play an important role in regulation and execution of apoptosis. Caspase 3 is composed of two subunits that are 17kDa and 12kDa, respectively.¹ The active enzyme is derived by proteolytic cleavage of a proenzyme called CPP32.¹

Specific Activity: Approximately 6,750 Units/mg of protein where 1 Unit = 1nmol of substrate (Caspase 3 chromogenic substrate, Ac-Asp-Glu-Val-Asp-pNA) hydrolyzed/minute.

Purity: >50% pure enzyme in the active (cleaved) confirmation as determined by SDS-PAGE followed by Coomassie Blue staining.

Formulation: 20µg of protein in 200µl phosphate buffered saline (PBS) containing 50% glycerol. Liquid at -20°C.

Storage and Stability: Stable for 6 months at -20°C from date of shipment. For maximum recovery of product, centrifuge original vial after thawing and prior to removing the cap.

**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing and Research Applications

Protease Assay: 0.05µg of this lot hydrolyzed a chromogenic substrate specific for Caspase 3. A previous lot of this product was shown to have no hydrolytic activity toward a fluorometric substrate specific for Caspase 1 (ICE).

Reference:

1. Nicholson, D., *et al.*, *Nature* **376**: 37-43, 1995.
2. Kothakota, S., *et al.*, *Science* **278**: 294-298, 1997.

Caspase 3 Assay

Stock Solutions:

1. Assay Buffer (AB): 50mM HEPES, pH 7.4, 100mM NaCl, 0.1% CHAPS, 1mM EDTA, 10% glycerol, 10mM dithiothreitol.
2. Caspase 3, free enzyme (Catalog # 14-264): Use 0.01-0.1µg per assay. Use AB to dilute the enzyme as needed.
3. Caspase 3 chromogenic substrate: Dissolve 2mg of substrate in 0.4ml of DMSO to prepare a 7.8mM stock solution. Immediately prior to use, dilute an aliquot to 1mM with AB. Use 30µl per assay, this yields a final substrate concentration of 300µM in the assay described below. Extinction Coefficient at 405nm: 9160cm⁻¹M⁻¹.

Assay Protocol:

1. Place 55µl of assay buffer into each well of a 96-well plate.
2. Add 15µl (0.01-0.1µg) of the diluted Caspase 3 stock.
3. Add **30µl of Caspase 3 chromogenic substrate**.
4. Continuously monitor the absorbance reading at A₄₀₅ for 5-60 minutes at 30°C.

$$\text{Specific Activity} = \frac{\Delta A \times \text{assay volume in liters} \times 10^9 \text{ Units/mole}}{\Delta t \text{ in minutes} \times 9160\text{cm}^{-1}\text{M}^{-1} \times \text{path length in cm} \times \text{mg of Caspase 3}} = \frac{\text{Units}}{\text{mg}}$$

The above expression may be simplified to:

$$\text{Specific Activity} = \frac{\Delta A \times V \times 109}{\Delta t \text{ in minutes} \times l \times m} = \frac{\text{Units}}{\text{mg}}$$

Where V = volume of reaction in µl
 l = path length in cm
 m = mass of Caspase in µg

For example, if in a 30 minute assay the absorbance changed 0.464 in a 100µl reaction volume using 0.05µg of Caspase 3 and the path length was 0.5cm, the specific activity = $\frac{0.465 \times 100 \times 109}{30 \times 0.5 \times 0.05} = 6,750 \frac{\text{Units}}{\text{mg}}$