

Caspase 3/Apopain/ CPP32/YAMA
(Histidine c-terminal tag, free enzyme)

Catalog # 14-264

Lot # 16377

Product Description: Recombinant full length protein containing a C-terminal histidine tag; expressed in *E. coli*. Caspase-3 (Apopain/YAMA) is a member of the ICE/CED3 family of cysteine proteases that play an important role in regulation and execution of apoptosis. Apopain/YAMA 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: 52,000Units/mg of protein where 1 Unit = 1nmol of substrate (Caspase 3/Apopain/ CPP32 Fluorometric Substrate, Catalog # 12-323) hydrolyzed/minute.

Purity: >75% pure enzyme in the active (cleaved) confirmation as determined by SDS-PAGE followed by Coomassie Blue staining.

Quantity and Formulation: **20ng** free protein in **200µl** of 0.05M phosphate buffered saline containing 50% glycerol.

Physical Form: Cold liquid.

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

Reference:

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

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing and Research Applications

Protease Assay: 0.01 μ g (10 μ l of a 1:100 dilution) of this lot hydrolyzed a fluorometric substrate specific for Apopain/YAMA. A previous lot of this product was shown to have no hydrolytic activity toward a fluorometric substrate specific for ICE (Interleukin 1 β Converting Enzyme).

Protease Protocol

Reagents:

1. Modified RIPA buffer, minus protease inhibitors: 50mM Tris-HCl, pH7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA.
2. Caspase 3 Fluorometric Substrate (Catalog # 12-323): Use at a final concentration of 5-50 μ M per 7 μ l.
3. Caspase 3/Apopain: Use at a final dilution of 0.01-1 μ g per 10 μ l.

Assay Protocol:

1. Turn on fluorometer for 15 minutes prior to starting the assay.
2. Place 1ml of modified RIPA buffer minus protease inhibitors into the fluorometer cuvette.
3. Add 7 μ l of fluorometric substrate.
4. Add 10 μ l of Caspase 3/Apopain.
5. Incubate for 5 minutes at room temperature.
6. Calculate activity using the following formula.

$$(FI/500\text{Unit/nmol}) \times (1/\text{time [min]}) \times (1/\text{Caspase 3 } [\mu\text{g}]) = \text{nmol/min } \mu\text{g} = \text{Unit activity}/\mu\text{g Caspase 3}$$

FI = units of fluorescence intensity.

1nmol = 500Units FI.

1Unit of activity = 1 nmol of Caspase Substrate hydrolyzed/minute.