

**Caspase 3 Activity Detection Kit**

**Catalog # 17-198**

**Lot # 16549**

**Kit Components**

**Caspase 3/Apopain/PPP32 Fluorometric Substrate**, Catalog # 12-323, Lot # 14521, see page two for more information.

One vial containing **5mg** lyophilized powder. MW = 694.

**Caspase 3/Apopain/PPP32/YAMA**, Catalog # 14-264, Lot # 16377, see page two for more information.

One vial containing **20µg** of protein in **200µl** of 0.05M phosphate buffered saline containing 50% glycerol.

Other components required but not included as part of this kit are:

- Modified RIPA Buffer
- reagent reservoirs
- pipettes and tips
- quartz cuvettes for fluorometry
- fluorometer
- stopwatch

**Lot Analysis**

**Quantity:** 130 assays per kit using 0.1µg of enzyme and 50µM substrate per assay.

**Storage and Stability:** Stable for 6 months at -20° from date of shipment.

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

### **Technical Information for Kit Components**

#### **Caspase3/Apopain/PPP32 Fluorometric Substrate (Ac-Asp-Glu-Val-Asp-amino-4-methyl-coumarin)**

**Background:** Caspase 3 (Apopain), which is derived from the proenzyme PPP32, is a member of the ICE (interleukin-1 $\beta$  converting enzyme) family of cysteine proteases. It hydrolyzes poly (ADP-ribose) polymerase (PARP) and appears necessary for apoptosis<sup>1</sup>. Synthetic Ac-Asp-Glu-Val-Asp-amino-methyl coumarin contains the PARP cleavage site, which is hydrolyzed by Caspase 3 to yield a species that fluoresces at 460nm<sup>1</sup>.

**Purity:** Greater than 95% by HPLC.

**Storage and Stability:** Lyophilized: 2 years at -20°C from date of shipment. Rehydrated: 4 months at -20°C. Dissolve the lyophilized powder in 1ml of DMSO to yield a 7.2mM stock.

#### **Caspase 3/Apopain/PPP32/YAMA (Histidine c-terminal tag, free enzyme)**

**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. Caspase 3 is composed of two subunits that are 17kDa and 12kDa, respectively.<sup>1</sup> The active enzyme is derived by proteolytic cleavage of a proenzyme called PPP32.<sup>1</sup>

**Specific Activity:** 52,000Units/mg of protein where 1 Unit = 1nmol of substrate (Caspase 3/Apopain/PPP32 Fluorometric Substrate, Catalog # 12-323) hydrolyzed/minute.

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

**Physical Form:** Cold liquid.

**Storage and Shelf Life:** 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.

#### **Reference:**

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

### Quality Control Testing and Research Applications

Protease Assay: 0.01µg (10µl of a 1µg/ml stock) of this lot hydrolyzed a fluorometric substrate specific for Caspase 3. A previous lot of this product was shown to have no hydrolytic activity toward a fluorometric substrate specific for ICE (Interleukin 1β Converting Enzyme).

#### Protease Assay

##### Reagents:

1. Modified RIPA buffer without protease inhibitors: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA.
2. Caspase 3/Apopain/PPP32 Fluorometric Substrate (Catalog # 12-323): Prepare a 0.72-7.2mM stock for use at a final concentration of 5-50µM. Dissolve 5mg of substrate in 1ml of DMSO to prepare a 7.2mM stock solution
3. Caspase 3/Apopain/PPP32/YAMA: Prepare a 1-10µg/ml stock for use at 0.01-0.1µg per assay.

##### Assay Protocol:

1. Turn on fluorometer for 15 minutes prior to starting the assay.
2. Set the excitation wavelength at 380nm and the emission (detection) wavelength at 460nm.
3. Place 1ml of modified RIPA buffer minus protease inhibitors into the fluorometer cuvette.
4. Add 7µl (5-50µM final concentration) of fluorometric substrate.
5. Add 0.01-0.1µg of Caspase 3 in 10µl.
6. Incubate for 5 minutes at room temperature.
7. Read the relative fluorescence of the sample.
8. Calculate activity using the following formula.

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

FI = units of fluorescence intensity.

1nmol = 500Units FI.

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