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## Certificate of Analysis

### Caspase 3 Activity Detection Kit

Catalog # 17-198

Lot # 29880

#### Kit Components

**Caspase 3 Fluorometric Substrate, (Ac-Asp-Glu-Val-Asp-AMC)**, Catalog # 12-323, Lot # 29881, see page two for more information. One vial containing **5mg** of lyophilized powder. MW = 674Da.

**Caspase 3 (recombinant protein expressed in *E. coli*)**, Catalog # 14-264, Lot # 26083, see page two for more information. One vial containing **20µg** of protein in **200µl** PBS containing 50% glycerol.

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

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#### Quality Control Testing

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

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

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

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

#### Reagents

- Modified RIPA Buffer

#### Equipment

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

## **Technical Information for Kit Components**

### **Caspase 3 Fluorometric Substrate (Ac-Asp-Glu-Val-Asp-AMC)**

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

**Application:** Use 5-50 $\mu$ M to assay Caspase 3 activity *in vitro* as described previously.<sup>1</sup>

**Physical Form:** Lyophilized powder. Stable for 2 years at -20°C. Dissolve lyophilized powder in 1ml of DMSO. Once dissolved, it is stable for 4 months at -20°C.

### **Caspase 3 (recombinant protein expressed in *E. coli*)**

**Product Description:** Recombinant full length protein containing a C-terminal histidine tag; expressed in *E. coli*. Caspase 3 is composed of two subunits that are 17kDa and 12kDa, respectively.<sup>1</sup>

**Specific Activity:** Approximately 6,750Units/mg of protein where 1 Unit = 1nmol of Caspase 3 Chromogenic Substrate, Catalog # 12-390) hydrolyzed/minute.

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

**Physical Form:** Liquid. 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.

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#### **General References:**

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

##### Stock Solutions:

1. Caspase 3 Fluorometric Substrate: Prepare a 0.72-7.2mM stock for use at a final concentration of 5-50 $\mu$ M. Dissolve 5mg of substrate in 1ml of DMSO to prepare a 7.2mM stock solution
2. Caspase 3: Prepare a 1-10 $\mu$ g/ml stock for use as a positive control at 0.01-0.1 $\mu$ g per assay.
3. Modified RIPA buffer without protease inhibitors: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA.

##### 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 7 $\mu$ l (5-50 $\mu$ M final concentration) of fluorometric substrate.
5. Add 10 $\mu$ l (0.01-0.1 $\mu$ g) of Caspase 3 (positive control) or a cell lysate sample and read immediately to determine relative fluorescence at time zero.
6. Incubate for 5 minutes at room temperature.
7. Read the relative fluorescence of the sample.
8. Calculate activity using the following formula.

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

FI = units of fluorescence intensity.

1nmol = 500Units FI.

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