
Certificate of Analysis

Caspase 3 Activity Detection Kit

Catalog # 17-198

Lot # 33524

Kit Components

Caspase 3 Fluorometric Substrate, (Ac-Asp-Glu-Val-Asp-AMC), Catalog # 12-323, Lot # 31858, 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 20 μ g of protein in 200 μ l PBS containing 50% glycerol.

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

Quality Control Testing

Protease Assay: 0.01 μ g (10 μ l of a 1 μ g/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.

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

Quantity: 130 assays per kit using 0.1 μ g of enzyme and 50 μ M 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: >90% by HPLC.

Application: Use 5-50 μ M to assay Caspase 3 activity *in vitro* as described previously.¹

Physical Form: Lyophilized powder. Lyophilized: Stable for 2 years at -20°C from date of shipment. Rehydrated: Stable for 4 months at -20°C. Rehydrate lyophilized powder in 1ml of DMSO. Aliquot rehydrated solution to avoid repeated freezing and thawing.

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.¹

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.

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 μ M. Dissolve 5mg of substrate in 1ml of DMSO to prepare a 7.2mM stock solution
2. Caspase 3: Prepare a 1-10 μ g/ml stock for use as a positive control at 0.01-0.1 μ 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, 10mM DTT added fresh.

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 μ l (5-50 μ M final concentration) of fluorometric substrate.
5. Add 10 μ l (0.01-0.1 μ 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.

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

FI = units of fluorescence intensity.

1nmol = 500Units FI.

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