#5723 Store at –20°C

Caspase-3 Activity Assay Kit

☑ 1 Kit
(200 assays)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Description: The Caspase-3 Activity Assay Kit is a fluorescent assay that detects the activity of caspase-3 in cell lysates. It contains a fluorogenic substrate (N-Acetyl-Asp-Glu-Val-Asp-7-amino-4-methylcoumarin or Ac-DEVD-AMC) for caspase-3. During the assay, activated caspase-3 cleaves this substrate between DEVD and AMC, generating highly fluorescent AMC that can be detected using a fluorescence reader with excitation at 380 nm and emission between 420 - 460 nm. Cleavage of the substrate only occurs in lysates of apoptotic cells; therefore, the amount of AMC produced is proportional to the number of apoptotic cells in the sample.

Background: Caspase-3 (CPP-32, Apoptain, Yama, SCA-1) is a critical executioner of apoptosis, as it is either partially or totally responsible for the proteolytic cleavage of many key proteins, such as the nuclear enzyme poly (ADP-ribose) polymerase (PARP) (1). Activation of caspase-3 requires proteolytic processing of its inactive zymogen into activated p17 and p12 fragments. Cleavage of caspase-3 requires the aspartic acid residue at the P1 position (2).

Caspase-7 (CMH-1, Mch3, ICE-LAP3) has been identified as a major contributor to the execution of apoptosis (3-6). Caspase-7, like caspase-3, is an effector caspase that is responsible for cleaving downstream substrates, such as PARP (3,5). During apoptosis, caspase-7 is activated by upstream caspases through proteolytic processsing at Asp23, Asp198, and Asp206, thereby producing the mature subunits (3,5). Similar to caspases-2 and -3, caspase-7 preferentially cleaves substrates following the recognition sequence DEVD (7).

Specificity/Sensitivity: Caspase-3 Activity Assay Kit detects fluorescent AMC dye produced from cleavage of Ac-DEVD-AMC by activated caspase-3 in apoptotic cells. This kit is expected to work in most species. Depending on the cell type and the incubation time applied in the assay, 0.5 - 2x10^5 cells/well (or 100 µg/well of total lysate protein) is sufficient for most experimental setups. For best results, cell number or lysate concentration titrations are recommended (see Figures 1 and 2). Because caspase-7 shares the same susbtrate sequence as caspase-3, this kit also detects caspase-7 activity.

Background References:

(1) Fernandes-Alnemri, T. et al. (1994) *J. Biol. Chem.* 269, 30761-30764.

(2) Nicholson, D. W. et al. (1995) *Nature* 376, 37-43.

(3) Fernandes-Alnemri, T. et al. (1995) *Cancer Res* 55, 6045-52.

(4) Duan, H. et al. (1996) *J Biol Chem* 271, 1621-5.

(5) Lippke, J.A. et al. (1996) *J Biol Chem* 271, 1825-8.

(6) Cohen, G.M. (1997) *Biochem J* 326 (Pt 1), 1-16.

(7) Thornberry, N.A. et al. (1997) *J Biol Chem* 272, 17907-11.

Products Included	Product Number	Quantity	Storage Temp
Ac-DEVD-AMC Fluorescent Substrate	11734	1 mg	-20°C
AMC (7-amino-4-methylcoumarin)	11735	250 µl	-20°C
PathScan® Sandwich ELISA Lysis Buffer (1X)	7018	30 ml	-20°C
Caspase Assay Buffer (2X)	11736	30 ml	-20°C
DTT (Dithiothreitol)	7016	192.8 mg	4°C

Important: Store DTT at -20C once in solution.
Note: This kit contains mixed storage components. Upon first use, please allow components to thaw and then store each component as indicated on individual component labels.

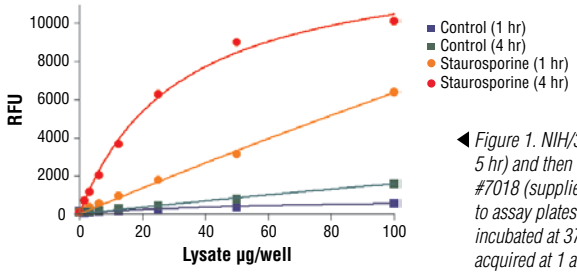

Figure 1. NIH/3T3 cells were treated with Staurosporine #9953 (5 µM, 5 hr) and then lysed in PathScan® Sandwich ELISA Lysis Buffer (1X) #7018 (supplied with kit). Various amounts of cell lysate were added to assay plates containing the substrate solution, and plates were incubated at 37°C in the dark. Relative fluorescent units (RFUs) were acquired at 1 and 4 hr.

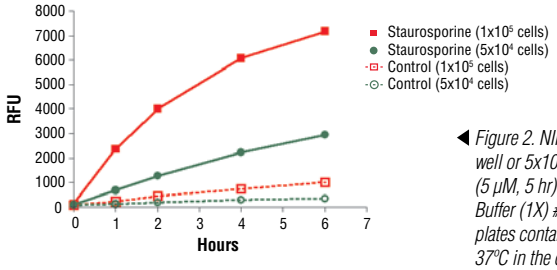

Figure 2. NIH/3T3 cells were seeded in a 96-well plate at 1x10^5 cells/well or 5x10^4 cells/well, and then treated with Staurosporine #9953 (5 µM, 5 hr) and then lysed in 30 µl PathScan® Sandwich ELISA Lysis Buffer (1X) #7018 (supplied with kit). Cell lysate was added to assay plates containing the substrate solution, and plates were incubated at 37°C in the dark. Relative fluorescent units (RFUs) were acquired at 0, 1, 2, 4, and 6 hr.

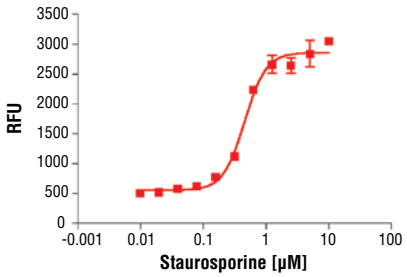

Figure 3. HeLa cells were seeded at 1x10^5 cells/well in a 96-well plate and incubated overnight. Cells were treated with various concentrations of Staurosporine #9953 (5 hr) and then lysed in 30 µl of PathScan® Sandwich ELISA Lysis Buffer (1X) #7018 (supplied with kit). Cell lysate was mixed with substrate solution and incubated at 37°C in the dark for 2 hr and relative fluorescent units (RFUs) were acquired.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Assay Protocol

A. Reagent Preparation

1. Reconstitute Ac-DEVD-AMC in 1 ml DMSO.
2. Thaw out reagents just before experiment.
3. Prepare 1M DTT (192.8 mg DTT #7016 1.12ml dH_2O). Make sure DTT crystals are completely in solution.

Important: Once in solution, store 1M DTT at -20°C.

Note: Precipitation may occur when reagents are stored at -20°C. Warm reagents to 37°C if necessary to dissolve precipitate.

4. Mix one part Assay buffer (2X) with one part dH_2O, and add DTT (1:200 dilution, final concentration of 5 mM) to make **1X assay buffer A.**
5. Dilute Ac-DEVD-AMC (1:40 dilution) in **1X assay buffer A** to make **substrate solution B.**

B. Cell Lysate Preparation: Collect lysate from 96-well plate

1. Plate cells in 96-well plate and incubate with respective test substance for appropriate time. Typical cell count is $5x10^4$ - $2x10^5$ cells/well.
2. Following treatment, spin plate at 300xg for 10 min, remove the medium, rinse cells with ice-cold PBS, spin plate at 300xg for 10 min, remove PBS.
3. Add 30 µl/well of cell lysis buffer #7018 and leave plate on ice for 5 min. (**NOTE:** Cell lysate plate can be stored at -80°C for future use.)

Collect lysate from petri dish:

a. Check cell adhesion following treatment. If cells detach from the plate or are only loosely attached to plate, proceed to step b; if cells are tightly adhered to plate, proceed to step c.
b. Rinse plate with existing medium to collect all cells in a centrifuge tube. Spin at 1000xg cpm for 5 min, remove supernatant, and add cell lysis buffer #7018 (0.5 ml/10 cm plate) to cell pellet. Pipette up and down a few times to break up the cells. Keep on ice and proceed to step d.
c. Rinse cells with ice-cold PBS, then add cell lysis buffer #7018 (0.5 ml/10 cm plate) to plate and leave on ice for 5 min. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice and proceed to step d.
d. Sonicate lysates on ice.
e. Microcentrifuge for 10 min at 4°C and transfer the supernatant to a tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

C. Caspase Activity Assay

1. Dilute cell lysate in **1X assay buffer A** to desired concentration (0.5 – 4 mg/ml is recommended). If cell lysates are from a 96-well plate, no dilution is necessary.
2. (Optional) Mix 25 µl of positive control AMC (supplied with kit) with 200 µl **1X assay buffer A** to serve as a positive control.
3. Mix 200 µl of **substrate solution B** and 25 µl lysate solution in a black plate appropriate for fluorescent assay.
   NOTE: We recommend reading the plate immediately and recording RFU reading at time 0 hr. This will help determine if there is significant change in RFU at the end of incubation.
   NOTE: This protocol has been tested in 384-well plate format, please adjust the volume proportionally based on the plate capacity. For example, if using 384-low volume plate, use 20 µl **substrate solution B** and 2.5 µl lysate.
4. Incubate plates at 37°C in the dark.
5. Read RFU on a fluorescence plate reader with excitation at 380 nm and emission at 420 – 460 nm.
   NOTE: We recommend reading plates after 1 hr incubation. If the signal is too weak, increase incubation period to observe significant change in signal strength. If significant increase is signal strength is not observed, more lysate may be necessary.