

Caspase-3 Activity Assay Kit

✓ 1 Kit
(200 assays)



Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 03/05/16

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 processing 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 - 2x10⁵ 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 substrate sequence as caspase-3, this kit also detects caspase-7 activity.

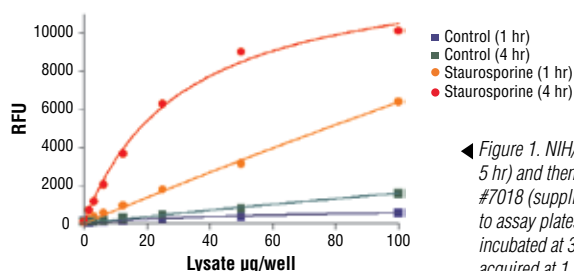
Background References:

- (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.

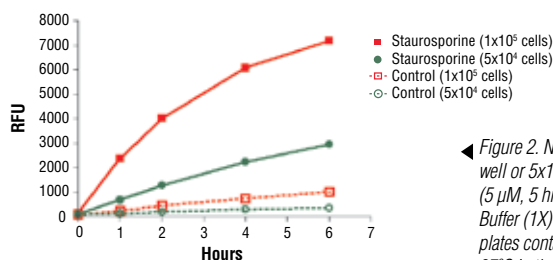
Products Included	Product Number	Quantity	Storage Temp
Ac-DEVD-AMC Fluorescent Substrate	11734	1 mg	-20°C
AMC (7-amino-4-methylcoumarin)	11735	250 µl	-20°C
PathScan® Sandwich ELISA Lysis Buffer (1X)	7018	30 ml	-20°C
Caspase Assay Buffer (2X)	11736	30 ml	-20°C
DTT (Dithiothreitol)	7016	192.8 mg	4°C

Important: Store DTT at -20°C once in solution.

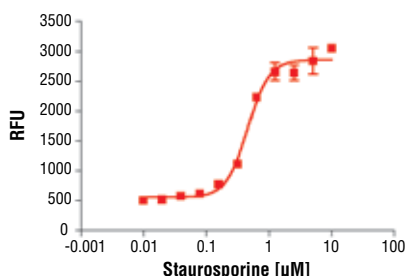
Note: This kit contains mixed storage components. Upon first use, please allow components to thaw and then store each component as indicated on individual component labels.



◀ 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 1x10⁵ cells/well or 5x10⁴ cells/well, and then treated with Staurosporine #9953 (5 µM, 5 hr) and then lysed in 30 µl of 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 1x10⁵ 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.

- (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.

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 dH₂O). 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 dH₂O, 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 5×10^4 - 2×10^5 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 μ l/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 μ l of positive control AMC (supplied with kit) with 200 μ l **1X assay buffer A** to serve as a positive control.
3. Mix 200 μ l of **substrate solution B** and 25 μ l 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 μ l **substrate solution B** and 2.5 μ l 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 in signal strength is not observed, more lysate may be necessary.



Material Safety Data Sheet (MSDS)

SDS# : 5723

Revision Date 2012-08-22
Version 2

1. PRODUCT AND COMPANY IDENTIFICATION

Product Name Caspase 3 Activity Assay Kit
Product Number 5723
Identified Uses For Research Use Only (RUO). Not intended for use in humans or animals.
Not intended for therapeutic or diagnostic procedures.

Manufacturer/Supplier Cell Signaling Technology, Inc.
3 Trask Lane
Danvers, MA 01923 USA
Phone #: 1-978-867-2300
Emergency Tel# 1-978-578-6737

2. HAZARDS IDENTIFICATION

Emergency Overview

OSHA Hazards

This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).



Warning!

GHS Classification

Skin irritation (Category 2)
Eye irritation (Category 2A)
Specific target organ toxicity –single exposure (Category 3)

Physical State

Kit box contains vials of liquids and solids.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous Ingredients

Please see the individual material safety data sheets which can be found on the CST website www.cellsignal.com/support/msds.html for hazard communication information specific to individual kit components contained in this product.

Component SKU	Kit Component Name
811734S	AC-DEVD-AMC
11735S	AMC (7-amino-4-methylcoumarin)
7081S	Pathscan ELISA Lysis Buffer (1X)
11736S	Caspase Assay Buffer (2X)
7016S	1M DTT

4. FIRST AID MEASURES

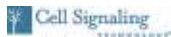
Eye Contact Rinse immediately with plenty of water. Get medical attention.
Skin Contact Rinse immediately with soap and plenty of water. Get medical attention.
Inhalation Move to fresh air. Get medical attention.
Ingestion Call Poison Control Center immediately. Never give anything by mouth to an unconscious person. Rinse mouth with water. Get medical attention.
Notes to physician Treat symptomatically.

5. FIRE FIGHTING MEASURES

Flash Point See Section 9. Physical and Chemical Properties
Suitable Extinguishing Media Use dry chemical

Cell Signaling Technology, Inc.
www.cellsignal.com

1



Material Safety Data Sheet (MSDS)

SDS# : 5723

Revision Date 2012-08-22
Version 2

Conditions/Materials to avoid No data available
Hazardous decomposition products No data available

11. TOXICOLOGICAL INFORMATION

Acute Toxicity

To the best of our knowledge, the chemical, physical and toxicological properties have not been fully investigated.

Potential Health Effects

Acute Toxicity
Eyes Causes eye irritation.
Skin May be harmful if absorbed through skin. Causes skin irritation
Inhalation May be harmful if inhaled. Causes respiratory tract irritation
Ingestion May be harmful if swallowed.

Chronic Effects

Carcinogenic effects No known significant effects or critical hazards.
Mutagenic effects No known significant effects or critical hazards.
Teratogenic effects No known significant effects or critical hazards.
Reproductive toxicity No known significant effects or critical hazards.
Sensitization May cause allergy or asthma symptoms or breathing difficulties if inhaled.

12. ECOLOGICAL INFORMATION

Ecotoxicity The environmental impact of this product has not been fully investigated.

Persistence and degradability Not available

Bioaccumulation Not available

Mobility Not available

13. DISPOSAL CONSIDERATIONS

Waste Disposal Methods: Dispose of in accordance with all applicable environmental laws and regulations.

14. TRANSPORT INFORMATION

IATA Not regulated as dangerous goods

DOT Not regulated as dangerous goods

MEX Not regulated as dangerous goods

15. REGULATORY INFORMATION

OSHA Hazards

This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).



Warning!

GHS Classification

Skin irritation (Category 2)
Eye irritation (Category 2A)
Specific target organ toxicity –single exposure (Category 3)

Hazard Statements

H315 Causes Skin Irritation

Cell Signaling Technology, Inc.
www.cellsignal.com

3



Material Safety Data Sheet (MSDS)

SDS# : 5723

Revision Date 2012-08-22
Version 2

Special exposure hazards No data available
Special protection for fire fighters Wear appropriate self-contained breathing apparatus and protective unit
Other information No data available

6. ACCIDENTAL RELEASE MEASURES

Personal precautions Do not touch or walk through spilled material. Wear personal protective equipment.
Environmental precautions Do not let product enter drains.
Clean up methods Soak up with absorbent material. Keep in suitable closed containers for disposal.
Other information See sections 12 and 13 for additional information.

7. HANDLING AND STORAGE

Safe handling advice Avoid contact with eyes and skin. Ensure adequate ventilation.
Storage condition Keep container tightly closed in a cool dry location.
Incompatibilities No data available
Specific end uses No data available

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Exposure Controls

Apply technical measures to comply with the occupational exposure limits.

Engineering Controls

Emergency eyewash and safety shower. Mechanical exhaust required.

Hygiene Measures

Do not eat, drink or smoke when handling product. Wash hands thoroughly after handling product. Wash contaminated clothing before reuse.

Personal Protective Equipment

Respiratory Protection: In case of insufficient ventilation wear suitable respiratory equipment.
Eye Protection: Safety glasses with side shields
Skin and body protection: Wear suitable protective clothing, protective shoes or boots.
Hand protection: Compatible chemical resistant gloves.

9. PHYSICAL AND CHEMICAL PROPERTIES

Information on basic physical and chemical properties

Physical State small vials of liquids and solids
Appearance no data available
Odor no data available
pH no data available
Water Solubility no data available
Melting point/freezing point no data available
Boiling point no data available
Evaporation rate no data available
Flammability no data available
Flash point no data available
Autoignition temperature no data available
Flammability limits in air no data available
Explosive properties no data available
VOC content no data available
Decomposition temperature no data available

10. STABILITY AND REACTIVITY

Stability

Stable under normal conditions

Cell Signaling Technology, Inc.
www.cellsignal.com

2



Material Safety Data Sheet (MSDS)

SDS# : 5723

Revision Date 2012-08-22
Version 2

H319 Causes serious eye irritation
H335 May cause respiratory irritation

Precautionary Statement

P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P264 Wash skin thoroughly after handling
P271 Use only outdoors or in a well-ventilated area.
P280 Wear protective gloves/ eye protection/ face protection
P302+P352 IF ON SKIN: Wash with plenty of soap and water.
P304+P340 IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes
P312 Call a POISON CENTER or doctor/ physician if you feel unwell.
P332+P313 If skin irritation occurs: Get medical advice/ attention.
P337+P313 If eye irritation persists: Get medical advice/ attention.
P362 Take off contaminated clothing and wash before reuse.
P403+P233 Store in a well-ventilated place. Keep container tightly closed.

International Inventories

TSCA (Ba) PAIR -
EINECS/ELINCS -
ENCS -
IECSC -
KECL -
PICCS -
AICS -
NZIoC -

U.S. Federal Regulations

SARA 302/304/311/312 hazardous chemicals: See individual kit component MSDS documents.

SARA 311/312 MSDS Distribution: See individual kit component MSDS documents.

U.S. State Regulations See individual kit component MSDS documents.

Massachusetts Right-to-Know No components are listed. CAS# 26093-31-2
Pennsylvania Right-to-Know 7-Amino-4-methylcoumarin CAS# 26093-31-2
New Jersey Right-to-Know 7-Amino-4-methylcoumarin

California Prop 65 Components

This product does not contain any chemicals known to the State of California to cause cancer, birth defects or any other reproductive harm.

Canada

This product as a whole is not controlled under WHMIS.

Kit components: 7-Amino-4-methylcoumarin CAS# 26093-31-2
D2B Toxic Material Causing Other Toxic Effects
Moderate skin irritant, Moderate respiratory irritant, Moderate eye irritant

16. OTHER INFORMATION

Revision date 2012-08-22

Revision Note ""Indicates updated section

Disclaimer

The information provided on this material safety data sheet is to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guide for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered as a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other material or in any process, unless specified in the text.

End of Material Safety Data Sheet

Cell Signaling Technology, Inc.
www.cellsignal.com

4