Caspase-3 Activity Assay Kit

**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 a highly fluorescent AMC that can be detected using a fluorescent 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 - 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 substrate sequence as caspase-3, this kit also detects caspase-7 activity.

**Background References:**

**Products Included**

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<th>Product Number</th>
<th>Quantity</th>
<th>Storage Temp</th>
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<tbody>
<tr>
<td>11734</td>
<td>1 mg</td>
<td>-20°C</td>
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<td>11735</td>
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<td>11736</td>
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</tr>
<tr>
<td>7016</td>
<td>192.8 mg</td>
<td>4°C</td>
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**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.

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**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 μM) 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.
**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 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.)
4. 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.
5. 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.
6. Sonicate lysates on ice.
7. 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.
1. PRODUCT AND COMPANY IDENTIFICATION

Product Name: Caspase 3 Activity Assay Kit
Product Number: D120
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 #: 1-877-663-2507

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 2)

Physical State
Nitrocellulose, mixed 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.

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
Cell Poison Control Center immediately. Never give anything by mouth to an unconscious person. Rinse mouth with water. Get medical attention.

Toxicologist:
Rinse symptomatically.

5. FIRE FIGHTING MEASURES

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

6. ACCIDENTAL RELEASE MEASURES

Cleaning methods
Use appropriate personal protective equipment.

7. HANDLING AND STORAGE

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. 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.

8. PHYSICAL AND CHEMICAL PROPERTIES

Information on basic physical and chemical properties
Physical State
Small scale solids and solids

Appearance
No data available

Odor
No data available

Odor Threshold Value
No data available

Odorama Value
No data available

Odorized Value
No data available

9. TOXICOLOGICAL INFORMATION

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

10. STABILITY AND REACTIVITY

Decomposition temperature
No data available

Flammability
No data available

Melting point/freezing point
No data available

Water Solubility
No data available

Boiling point
No data available

Odor
No data available

11. ECOTOLOGY INFORMATION

Toxicity
The environmental impact of the product has not been fully investigated.

Persistence and degradability
Not available

Bioaccumulation
Not available

Mobility
Not available

12. DISPOSAL CONSIDERATIONS

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

13. TRANSPORT INFORMATION

IATA
Not regulated as dangerous goods

DOT
Not regulated as dangerous goods

Mex
Not regulated as dangerous goods

14. REGULATORY INFORMATION

OSHA Hazards
This material is considered hazardous by the OSHA Hazard Communication Standards (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
W215 Causes skin irritation