Caspase-3/7 Cell-Based Activity Assay Kit

Item No. 702790

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1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -80°C kit. After opening the kit, store individual components as stated below.

Item Number	Item	Quantity	Storage
10010210	Caspase-3/7 Inhibitor N-Ac-DEVD-CHO	1 vial/40 µl	-20°C
400702	Caspase-3/7 Substrate Ac-DEVD-AFC	1 vial/100 µl	-20°C
10010209	Active Caspase-3 Positive Control	1 vial/10 µl	-80°C
700416	DTT (1 M) Assay Reagent	1 vial/1 ml	-20°C
10009322	Cell-Based Assay Buffer Tablet	1 tablet	RT
10010215	Cell-Based Assay Lysis Buffer	1 vial/10 ml	RT
601772	Staurosporine Apoptosis Inducer	1 vial/100 µl	-20°C
400017	96-Well Solid Plate (black)	1 plate	RT
400023	Foil Plate Cover	1 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

This kit may not perform as described if any reagent or procedure is required or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section, on page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring fluorescence with excitation and emission wavelengths of 400±10 and 505±10 nm, respectively
2. Adjustable pipettes; multichannel or repeating pipettor recommended
3. A source of pure water; glass-distilled water or HPLC-grade water is acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
4. Materials for cell culture (cell culture plate, media, DMSO, serum, *etc.*)
5. An orbital microplate shaker

INTRODUCTION

Background

Caspases are a family of aspartate-specific cysteine proteases that are key mediators of apoptosis and inflammation but also have roles in pyroptosis, necroptosis, and autophagy.[1-4] Caspases participating in apoptosis are further classified as either initiator caspases (caspase-2, -8, -9 and -10) or executioner/effector caspases (caspase-3, -6 and -7). The precursor form of all caspases is composed of a pro-domain, which must be cleaved to activate the caspase, and large and small catalytic subunits, which associate into a tetramer to form the active enzyme. Caspase-3 and -7 are ubiquitously expressed and can be activated through the extrinsic or intrinsic apoptotic pathways *via* cleavage by caspase-8 or -9, respectively, or the granzyme B pathway.[5-7] The classical substrate cleavage site for both caspase-3 and -7 is Asp-Glu-Val-Asp (DEVD), with cleavage following the second aspartate.[1] These caspases act on several substrates, including poly(ADP-ribose) polymerase (PARP), DNA protein kinase (DNA-PK), growth arrest-specific protein 2 (GAS2), and procaspase-6.[1] The activation of these caspases is the functional endpoint of the apoptotic cascade and is used as a marker of apoptosis.[7-9] Caspase-3 and -7 are therapeutic targets for multiple diseases such as cancer, heart failure, and neurodegenerative diseases.

About This Assay

Cayman's Caspase-3/7 Cell-Based Activity Assay Kit provides a convenient method of detecting caspase-3 and caspase-7 activity in cell lysates. Activity is monitored using a caspase-3/7 specific substrate, N-Ac-DEVD-AFC. Cleavage by the active enzyme(s) generates a highly fluorescent product that can be measured using excitation and emission wavelengths of 400 and 505 nm, respectively. Active caspase-3 is included in the kit as a positive control and staurosporine is included to induce apoptosis. The caspase-3/7 inhibitor, N-Ac-DEVD-CHO, is included as a control to confirm the specificity of the fluorescence signal to the activity of the enzymes.

ASSAY PROTOCOL

Reagent Preparation

1. **Cell-Based Assay Buffer Tablet – (Item No. 10009322)**

   Dissolve the Cell-Based Assay Buffer Tablet in 100 ml of pure water. This buffer will be stable for at least one year when stored at room temperature.

2. **Cell-Based Assay Lysis Buffer – (Item No. 10010215)**

   This vial contains 10 ml of Cell-Based Assay Lysis Buffer. It is ready to use as supplied. This buffer will be stable for at least six months when stored at room temperature.

3. **Caspase-3/7 Substrate Ac-DEVD-AFC – (Item No. 400702)**

   This vial contains 100 µl of Caspase-3/7 Substrate N-Ac-DEVD-AFC. It is used to prepare the Substrate Solution. If all of the Caspase-3/7 Substrate N-Ac-DEVD-AFC will not be used at one time, aliquot and store at -20°C, where it will be stable for three months. Limit freeze-thaw cycles to two.

4. **DTT (1M) Assay Reagent – (Item No. 700416)**

   This vial contains 1 ml of 1 M DTT. It is used to prepare the Substrate Solution. If all of the DTT will not be used at one time, aliquot and store at -20°C, where it will be stable for three months. Limit freeze-thaw cycles to one.

5. **Active Caspase-3 Positive Control – (Item No. 10010209)**

   This vial contains 10 µl of recombinant human caspase-3. The enzyme should be thawed on ice and gently mixed prior to dilution. Do not vortex. To dilute the enzyme, combine 5 µl of Active Caspase-3 Positive Control with 495 µl of Cell-Based Assay Buffer and mix gently. It is recommended that the enzyme be diluted immediately prior to performing the assay. The remaining undiluted enzyme can be stored at -80°C, where it will be stable for three months. Limit freeze-thaw cycles to two.

6. **Staurosporine Apoptosis Inducer – (Item No. 601772)**

   This vial contains 100 µl of 1 mM Staurosporine Apoptosis Inducer in DMSO. Dilute Staurosporine Apoptosis Inducer in cell culture medium to use as a positive control. Concentrations and incubation times required to activate caspase-3/7 will vary depending on cell type; treatment with 5 µM staurosporine for 3 hours at 37°C may be sufficient for some cell types. If all of the Staurosporine Apoptosis Inducer will not be used at one time, aliquot the undiluted solution and store at -20°C, where it will be stable for three months. Limit freeze-thaw cycles to two.

7. **Caspase-3/7 Inhibitor N-Ac-DEVD-CHO – (Item No. 10010210)**

   This vial contains 40 µl of 4 mM Caspase-3/7 Inhibitor N-Ac-DEVD-CHO in DMSO. Dilute 5 µl of the inhibitor with 45 µl of Cell-Based Assay Buffer. This is a sufficient volume to inhibit four wells to test substrate specificity. If all of the Caspase-3/7 Inhibitor N-Ac-DEVD-CHO will not be used at one time, aliquot the undiluted inhibitor and store at -20°C where it will be stable for at least six months.

8. **Substrate Solution**

   To prepare the Substrate Solution, combine 100 µl of Caspase-3/7 Substrate Ac-DEVD-AFC, 400 µl of DTT (1M) Assay Reagent, and 9.5 ml of Cell-Based Assay Buffer. This is a sufficient volume to assay 96 wells. Scale down as needed. The Substrate Solution will be stable for 30 minutes at room temperature.

Plate Set Up

There is no specific pattern for using the wells on the plate. It is recommended that three wells be designated for the Active Caspase-3 Positive Control. It is suggested that each sample be assayed in triplicate and that the contents of each well are recorded on the template sheet provided on page 18. A typical layout of samples to be measured in triplicate is provided below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	B	B	6	6	6	14	14	14	22	22	22
B	PC	PC	PC	7	7	7	15	15	15	23	23	23
C	PCI	PCI	PCI	8	8	8	16	16	16	24	24	24
D	1	1	1	9	9	9	17	17	17	25	25	25
E	2	2	2	10	10	10	18	18	18	26	26	26
F	3	3	3	11	11	11	19	19	19	27	27	27
G	4	4	4	12	12	12	20	20	20	28	28	28
H	5	5	5	13	13	13	21	21	21	29	29	29

B = Background Wells
PC = Positive Control Wells
PCI = Positive Control + Inhibitor Wells
1-29 = Sample ± Inhibitor Wells

Figure 1. Sample plate format

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 200 µl in all of the wells.
- All reagents should be prepared as described above. The Active Caspase-3 Positive Control should be kept on ice and all other reagents should be kept at room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the samples be assayed in triplicate, but it is at the user's discretion to do so.
- 29 samples can be assayed in triplicate or 45 in duplicate.
- The assay is performed at 37°C.
- Monitor the fluorescence with excitation and emission wavelengths of 400±10 and 505±10 nm, respectively.

Performing the Assay

Optimal seeding density will be dependent on cell type and length of treatment. Overcrowding cells may result in high, non-specific fluorescence. It is recommended that appropriate vehicle controls be included for all test compounds, including the optional treatment with Staurosprine Apoptosis Inducer.

1. Culture cells in a 96-well plate and treat with test compounds and vehicle controls following typical experimental procedures. Perform each treatment in six replicates where three of the resulting cell lysates will be added to the **Sample Wells** and the other three to the **Sample + Inhibitor Wells**.
   a. It is recommended that adherent cells are cultured to 80% confluence prior to use in the assay.
   b. It is recommended that suspension cells reach a density of 5 x 10^4 to 5 x 10^5 prior to use in the assay.
2. Centrifuge the plate at 800 x g for 5 minutes and carefully aspirate the culture medium.
3. Gently wash the cells with 200 µl/well of Cell-Based Assay Buffer.
4. Centrifuge the plate at 800 x g for 5 minutes and carefully aspirate the buffer.
5. Add 100 µl of Cell-Based Assay Lysis Buffer to each well.
6. Incubate with gentle shaking on an orbital shaker for 30 minutes at room temperature.

7. Following the table below, add the appropriate amount of reagents to the designated wells in the supplied 96-well plate (Item No. 400017). Directly transfer 90 µl of cell lysates from step 6 to the **Sample Wells** and **Sample + Inhibitor Wells**.

Reagent	Background Wells	Positive Control Wells	Positive Control + Inhibitor Wells	Sample Wells	Sample + Inhibitor Wells
Cell-Based Assay Buffer	100 µl	10 µl	--	10 µl	--
Diluted Active Caspase-3 Positive Control	--	90 µl	90 µl	--	--
Diluted Caspase-3/7 Inhibitor N-Ac-DEVD-CHO	--	--	10 µl	--	10 µl
Cell Lysate	--	--	--	90 µl	90 µl

Table 1. Pipetting summary

8. Initiate the reaction with 100 µl of the Substrate Solution (see **Reagent Preparation**, page 8) and mix gently by pipetting up and down four to five times. Careful mixing will prevent the formation of bubbles in the wells.
9. Cover the plate with foil and incubate at 37°C for 60 minutes.
10. Read the fluorescence using excitation and emission wavelengths of 400±10 and 505±10 nm, respectively.

ANALYSIS

Performance Characteristics

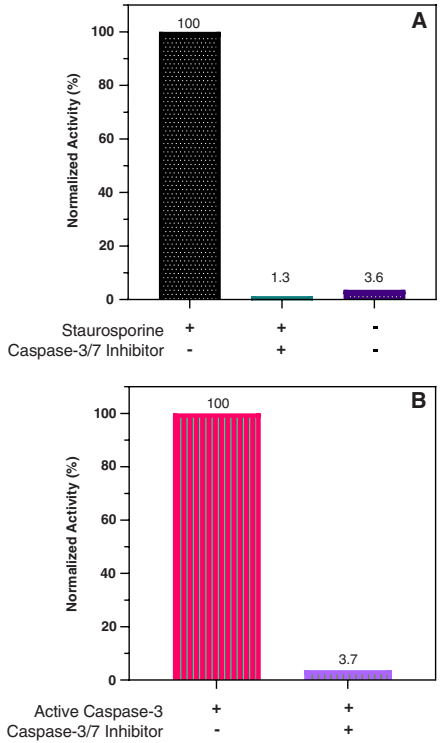


Figure 2. Activity of caspase-3/7. Panel A) Apoptosis was induced in Jurkat cells (2.5 x 10^5 cells/well) by treatment with 5 µM Staurosprine Apoptosis Inducer for 3 hours at 37°C; cell lysates were assayed for caspase-3/7 activity following the protocol on page 11. Panel B) Active Caspase-3 Positive Control was inhibited by the inhibitor control, Caspase-3/7 Inhibitor N-Ac-DEVD-CHO.

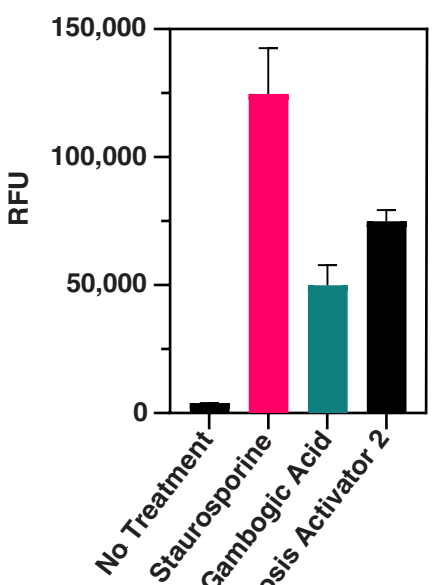


Figure 3. Caspase-3/7 activation using various apoptosis activators. Apoptosis was induced in Jurkat cells (2.5 x 10^5 cells/well) by treatment with 5 µM staurosporine, 2.5 µM Gambogic Acid*, or 500 µM Apoptosis Activator 2* and samples were assayed for caspase-3/7 activity using the Caspase-3/7 Cell-Based Activity Assay Kit. **Gambogic Acid (Item No. 14761) and Apoptosis Activator 2 (Item No. 10004176) are available for purchase from Cayman.*

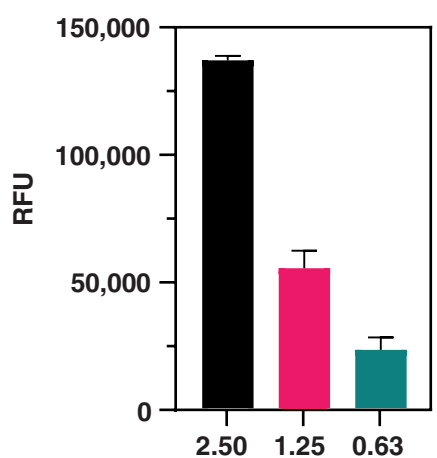


Figure 4. Caspase-3/7 activity across different cell densities. Caspase-3/7 activity was measured in Jurkat cells at different cell densities after treatment with 5 µM straurosporine for 3 hours at 37°C.

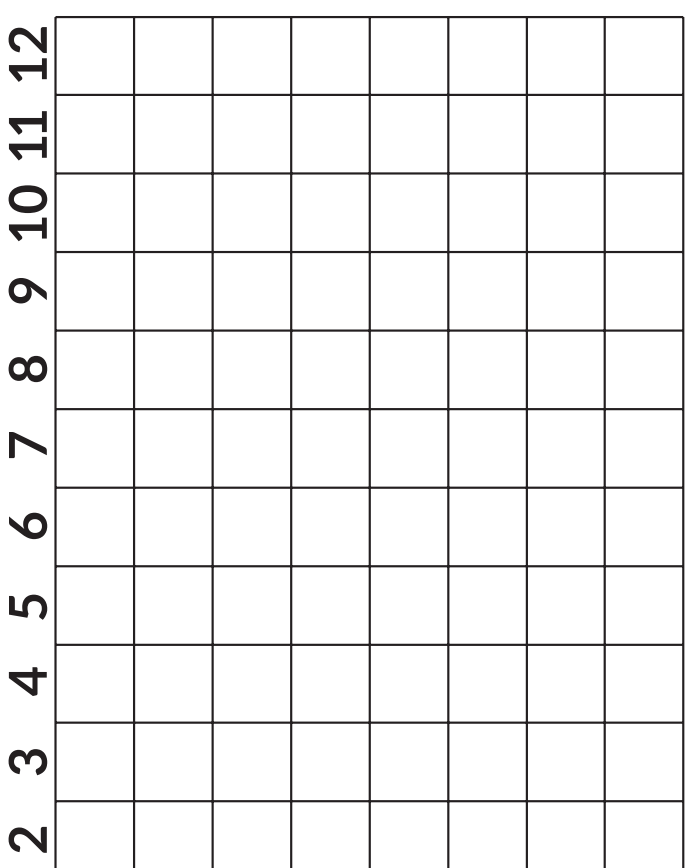
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/ triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
High readings in all wells	A. Phenol red residue in the wells B. Cell density is too high	A. Carefully aspirate all of the culture medium and wash the wells with the assay buffer thoroughly B. Plate cells more sparsely
Erratic response curve of compound treatments	A. Cells lost from wells during processing B. Unequal number of cells in each well	A. Increase replicates B. Use only healthy cells at the beginning of each experiment C. Make sure each well contains the same number of cells

References

1. Cohen, G.M. Caspases: The executioners of apoptosis. *Biochem. J.* **326(Pt 1)**, 1-16 (2007).
2. McIlwain,D.R., Berger,T., and Mak,T.W. Caspase functions in cell death and disease. *Cold Spring Harb. Perspect. Biol.* **5(4)**, a008656 (2013).
3. Sun, G. Death and survival from executioner caspase activation. *Semin. Cell Dev. Biol.* **156**, 66-73 (2024).
4. Sahoo, G., Samal, D., Khandayataray, P., *et al.* A review on caspases: Key regulators of biological activities and apoptosis. *Mol. Neurobiol.* **60(10)**, 5805-5837 (2023).
5. Fernandes-Alnemri, T., Litwack, G., and Alnemri, E.S. CPP32, a novel human apoptotic protein with homology to *Caenorhabditis elegans* cell death protein Ced-3 and mammalian interleukin-1β-converting enzyme. *J. Biol. Chem.* **269(49)**, 30761-30764 (1994).
6. Juna, T.S-C., McNiece, I.K., Argento, J.M., *et al.* Identification and mapping of *Casp7*, a cysteine protease resembling CPP32β, interleukin-1β converting enzyme, and CED-3. *Genomics* **40(1)**, 86-93 (1997).
7. Ghavami, S., Hashemi, M., Ande, S.R., *et al.* Apoptosis and cancer: Mutations within caspase genes. *J. Med. Genet.* **46(8)**, 497-510 (2009).
8. Yang, B., Ye, D., Wang, Y. Caspase-3 as a therapeutic target for heart failure. *Expert Opin. Ther. Targets* **17(3)**, 255-263 (2013).
9. Hartmann, A., Hunot, S., Michel, P.P., *et al.* Caspase-3: A vulnerability factor and final effector in apoptotic death of dopaminergic neurons in Parkinson's disease. *Proc. Natl. Acad. Sci. USA* **97(6)**, 2875-2880 (2000).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.