Caspase-3/7 Fluorescence Assay Kit

Item No. 10009135

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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	100 Tests Quantity/Size	Storage
10010208	Caspase-3/7 Substrate N-Ac-DEVD-N'-MC-R110	1 vial/100 µl	-20°C
10010209	Active Caspase-3 Positive Control	1 vial/10 µl	-80°C
10010210	Caspase-3/7 Inhibitor N-Ac-DEVD-CHO	1 vial/40 µl	-20°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

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.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888
Fax: 734-971-3641
Email: techserv@caymanchem.com
Hours: M-F 8:00 AM to 5:30 PM EST

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. Primary cells or a cell line and apoptotic treatments of choice
2. A 96-well plate for culturing cells
3. A black 96-well plate to use for the assay
4. A 96-well fluorescence plate reader capable of excitation at 485 nm and emission at 535 nm
5. A plate centrifuge

INTRODUCTION

Background

Caspases are members of the aspartate-specific cysteinyl protease family and are involved in regulation of apoptosis and inflammation.[1] Caspases participating in apoptosis are further classified as either initiator caspases (caspase-2, -8, -9 and -10) or effector caspases (caspase-3, -6 and -7).[2] Different caspases have distinct but overlapping cleavage specificities. For example, the classical cleavage site for both caspase-3 and -7 is Asp-Glu-Val-Asp (DEVD), with cleavage following the second Asp. Apoptosis occurs by three different pathways: (i) ligation of death receptors, (ii) cellular stress, or (iii) the granzyme B pathway, and all three lead to the proteolytic activation of the effector caspases.[3] Thus the activation of caspase-3 and -7 is the functional end-point of the apoptotic cascade, and is used as both a marker of apoptosis and a therapeutic target for overcoming apoptotic resistance in cancer.

About This Assay

Cayman's Caspase-3/7 Fluorescence Assay Kit employs a specific substrate, N-Ac-DEVD-N'-MC-R110, which upon cleavage by active caspase-3 or caspase-7, generates a highly fluorescent product that can be measured using excitation and emission wavelengths of 485 and 535 nm, respectively. The kit is simple to use and can be easily adapted to high-throughput screening for therapeutic compounds regulating activation of caspase-3 or -7. Active caspase-3 is included in the kit for use as a positive control. The caspase-3/7 inhibitor, N-Ac-DEVD-CHO, is also included in the kit for verifying the specificity of the substrate.

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Assay Buffer Preparation**

   Dissolve the Cell-Based Assay Buffer Tablet (Item No. 10009322) in 100 ml of distilled water. This buffer should be stable for approximately one year at room temperature.

2. **Caspase-3/7 Substrate Solution**

   To prepare enough substrate solution for one 96-well plate, combine 100 µl of the Caspase-3/7 Substrate N-Ac-DEVD-N'-MC-R110 (Item No. 10010208), 400 µl of DTT (1 M) Assay Reagent (Item No. 700416), and 9.5 ml of Assay Buffer. If you are not using an entire 96-well plate, adjust volumes accordingly.

   If you are not using all of the Caspase-3/7 Substrate stock at one time, we recommend that you make small aliquots and store them at -20°C.

3. **Active Caspase-3 Positive Control**

   One vial (Item No. 10010209) contains 10 µl of active caspase-3. To use as a positive control, dilute the contents 1:500-1:1,000 in Assay Buffer (prepared above).

4. **Caspase-3/7 Inhibitor Solution**

   Dilute the Caspase-3/7 Inhibitor N-Ac-DEVD-CHO (Item No. 10010210) 1:10 in Assay Buffer prior to use.

   If you are not using all of the Caspase-3/7 Inhibitor stock at one time, we recommend that you make small aliquots and store them at -20°C.

ASSAY PROTOCOL

Cell Set Up

1. Seed cells in a 96-well plate at a density of 5 x 10^4 to 5 x 10^5 cells/well in the appropriate culture medium and treat according to your experimental protocol.
2. It is critical that cells are not seeded too densely, as this may result in high nonspecific fluorescence. Actual seeding density will depend on cell type and should be optimized.
3. We recommend performing all treatments in triplicate.
4. Caspase activity is typically seen within a few hours of treatment, but exact timing must be determined empirically in your system.

Performing the Assay (Perform all steps at room temperature)

1. Centrifuge the plate in a plate centrifuge at 800 x g for five minutes.
2. Aspirate the culture medium.
3. Add 200 µl of Assay Buffer to each well and centrifuge the plate at 800 x g for five minutes.
4. Aspirate the supernatant.
5. Add 100 µl of Cell-Based Assay Lysis Buffer (Item No. 10010215) to each well.
6. Incubate with gentle shaking on an orbital shaker for 30 minutes at room temperature.

7. Centrifuge the plate at 800 x g for 10 minutes. Transfer 90 µl of the supernatant from each well to a corresponding well in a new black 96-well plate. Add 10 µl of Assay Buffer (sample activity measurment) or 10 µl of the Caspase-3/7 Inhibitor Solution (to test assay specificity) to appropriate wells.
8. Add 100 µl of Active Caspase-3 Positive Control into corresponding wells of the black plate.
9. Add 100 µl of the Caspase-3/7 Substrate Solution to each well and incubate the plate at 37°C for 30-90 minutes.
10. Read the fluorescence intensity of each well (excitation = 485 nm; emission = 535 nm).

ANALYSIS

Performance Characteristics

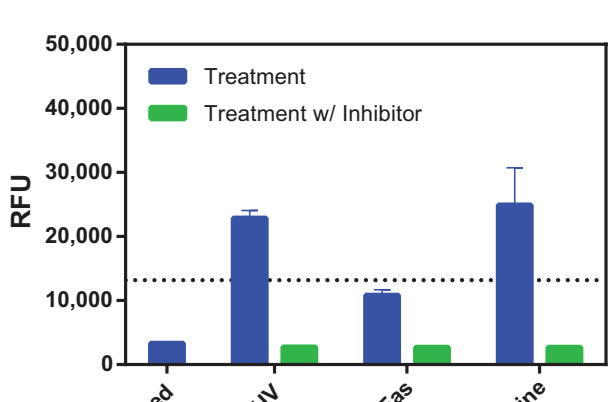

Figure 1. Activation of caspase-3/7 in Jurkat cells. Jurkat cells were seeded in a 96-well plate in 100 µl of culture medium at a density of 5 x 10^5 cells/well and treated with either (i) ultraviolet light (UV; 200 mJ/cm^2) (ii) anti-Fas antibody CH-11 (50 ng/ml) or (iii) staurosporine (5 µM) and incubated for three hours in a 37°C incubator before being processed for measurement of caspase-3/7 activity. The dotted line indicates the relative fluorescence of the positive control diluted 1:1,000 in Assay Buffer.

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/ triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Use a repeat pipettor to improve pipetting accuracy B. Carefully tap the side of the plate with your finger to remove bubbles. Be careful not to splash the contents of the wells
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 the experiment C. Make sure each well contains the same number of cells

References

1. McIlwain, D.R., Berger, T., and Mak, T.W. Caspase functions in cell death and disease. *Cold Spring Harb. Perspect. Biol.* **5(4)**, (2013).
2. Shi, Y. Mechanisms of caspase activation and inhibition during apoptosis *Mol. Cell.* **9(3)**, 459-470 (2002).
3. Ghavami, S., Hashemi, M., Ande, S.R., *et al.* Apoptosis and cancer: Mutations within caspase genes. *J. Med. Genet.* **46(8)**, 497-510 (2009).

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.