CaspGLOW™ Fluorescein Staining Kit

Item No. 602020

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GENERAL INFORMATION

Materials Supplied

Item Number	Item Name	Quantity/Size	Storage Temperature
400509	Fluorescein-VAD-FMK	1 vial/100 µl	-20°C
400510	Z-VAD-FMK	1 vial/10 µl	-20°C
10009322	Cell-Based Assay Buffer Tablet	1 vial/2 tablets	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 replaced 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

Fluorescein-VAD-FMK and Z-VAD-FMK will be stable for up to one year when stored at -20°C. Once reconstituted, the Cell-Based Assay Buffer will be stable for up to one year when stored at room temperature.

Materials Needed But Not Supplied

1. A CO_2 incubator set to 37°C
2. A fluorescence microscope, plate reader, or flow cytometer capable of measuring fluorescence with excitation and emission wavelengths of 485 and 535 nm, respectively
3. A source of pure water; glass-distilled, deionized, or ultrapure water is acceptable
4. Microcentrifuge
5. Microcentrifuge tubes
6. Microscope slides and coverslips
7. Black 96-well plate(s)

INTRODUCTION

Background

Caspases are a family of cysteinyl aspartic proteases whose members are involved in the regulation of apoptosis and inflammation.[1] Caspases are synthesized as inactive precursors containing a short or long prodomain, depending on the type of caspase, and a protease domain, which is composed of a large and small catalytic subunit that are cleaved apart and subsequently associate into a tetramer with other subunits to form the active enzyme.[1,2] The caspases involved in apoptosis are categorized as initiator caspases, which are activated by oligomerization followed by autoproteolysis, and effector caspases, which can be activated either by initiator caspases or other proteases.[1,3] Activation of initiator caspases is induced by apoptotic signals, including DNA damage, growth factor withdrawal, and γ-irradiation, and facilitated by apoptosis adapter proteins.[4] Caspases are potential therapeutic targets for diseases with dysregulated levels of apoptosis, including neurodegenerative and autoimmune diseases, stroke, and cancer.[5,6] Although this CaspGLOW™ staining method is commonly used for the detection of apoptosis, other forms of cell death may activate caspases and be detected using this kit.

About This Assay

Cayman's CaspGLOW™ Fluorescein Active Caspase Staining Kit provides a convenient and rapid tool to assess caspase activation in live cells with validated protocols for fluorescence microscopy, fluorimetry, and flow cytometry. Cayman's CaspGLOW™ Fluorescein Active Caspase Staining Kit provides 100 µl of dye, which is a sufficient volume to analyze up to 96 samples, and includes a caspase inhibitor as a negative control.

Principle Of This Assay

Upon activation by appropriate stimuli, cascading signaling events result in the dimerization, cleavage, and activation of caspases, leading to apoptosis and eventual cell death. Fluorescein-VAD-FMK (CaspGLOW™) will only exhibit a strong fluorescence signal upon binding to activated caspases. Cells that do not contain activated caspases will not retain the probe, leading to a much weaker fluorescence signal. Non-apoptotic cells will not retain the probe, leading to a much weaker fluorescence signal.

PRE-ASSAY PREPARATION

Buffer Preparation

Cell-Based Assay Buffer

Add one Cell-Based Assay Buffer Tablet (Item No. 10009322) to 100 ml of pure water and allow the tablet to dissolve. The reconstituted Cell-Based Assay Buffer will be stable for up to one year when stored at room temperature.

ASSAY PROTOCOL

Plate Set Up

It is recommended that each experiment include a negative control not stained with Fluorescein-VAD-FMK and a group treated with the included pan-caspase inhibitor Z-VAD-FMK to block activation of caspases. There is no recommended plate set up for analysis of samples.

Performing the Assay

1. Induce apoptosis in cultured cells (1 x 10^6/ml) using the desired method. Concurrently, include a control culture that does not undergo apoptosis. An additional negative control can be prepared by adding the pan-caspase inhibitor Z-VAD-FMK (Item No. 400510) at 1 µl/ml of culture media to inhibit activation of caspases.
2. After induction of apoptosis, aliquot 300 µl of induced or control cultured cells into microcentrifuge tubes.
3. Add 1 µl of Fluorescein-VAD-FMK (Item No. 400509) to each tube and pipette to mix. An unlabeled control can be included by adding 1 µl of DMSO to 300 µl of cell suspension. Incubate at 37°C in an incubator with 5% CO_2 for 30 minutes to one hour.
4. Centrifuge cells at 500 x g for 5 minutes and remove the supernatant.
5. Resuspend cells in 500 µl of Cell-Based Assay Buffer, and repeat the centrifugation step. Remove the supernatant. Perform one additional wash with 500 µl of Cell-Based Assay Buffer.
6. Proceed to step 8, 9, or 10 depending on the preferred method of analysis.
7. Flow cytometry: Resuspend cells in 300 µl of Cell-Based Assay Buffer and keep on ice. Analyze by flow cytometry using the FL-1 channel (FITC or similar excitation).
8. Fluorescence microscopy: Resuspend cells in 100 µl of Cell-Based Assay Buffer. Place an aliquot of the cell suspension on a microscope slide and cover using a coverslip. Observe the cells using a FITC/GFP filter. Cells with active caspases will have bright green fluorescence, while caspase-negative cells will show much weaker fluorescence.
9. Fluorescence plate reader: Resuspend cells in 100 µl of Cell-Based Assay Buffer and transfer the suspension to a black 96-well plate. Measure the fluorescence intensity at excitation/emission of 485/535 nm.

Performance Characteristics

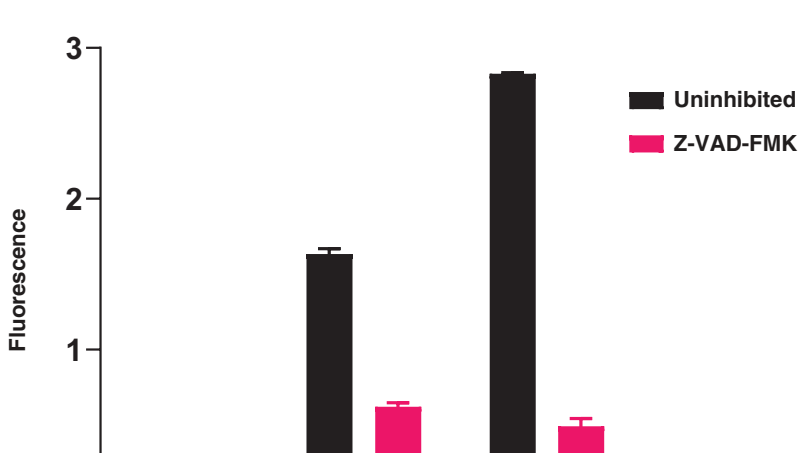

Figure 1. Caspase activation in A549 cells and inhibition by the negative control caspase inhibitor Z-VAD-FMK. A549 adenocarcinoma cells were treated with Apoptosis Activator 2 (Item No. 10004176) for 3.5 hours, followed by a 1-hour incubation with Fluorescein-VAD-FMK. Cells were washed as described in the **Performing the Assay** section (see page 8) and data acquired using the plate reader protocol. Cells incubated with apoptosis activator 2 displayed a dose-dependent fluorescence response, which was eliminated by concurrent administration of the pan-caspase inhibitor Z-VAD-FMK.

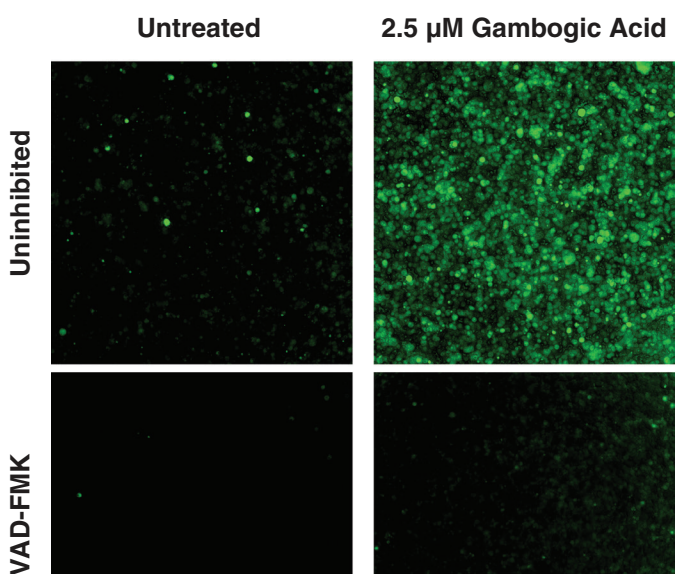

Figure 2. Caspase activation in HEK293T cells and inhibition by the negative control caspase inhibitor Z-VAD-FMK. HEK293T cells were treated with 2.5 µM Gambogic Acid (Item No. 14761) for 3 hours, followed by a 1-hour incubation with Fluorescein-VAD-FMK. Cells were washed as described in the **Performing the Assay** section and imaged using a fluorescence microscope imaging system. Cells incubated with gambogic acid contained active caspases leading to accumulation of the probe and increased cell-associated fluorescence. Z-VAD-FMK inhibited caspase activation by gambogic acid, yielding a lower fluorescent signal.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
High background	A. Cell density is too high B. Insufficient washing C. Cells incubated with dye for extended time period	A. Use suggested cell density during staining B. Use provided wash buffer as described in protocol C. Incubate cells for appropriate time
Low or absent signal	A. Cells did not undergo apoptosis B. Low cell density C. Incorrect equipment settings	A. Determine optimal experimental settings for apoptosis induction B. Use suggested cell density during staining C. Use suggested excitation/ emission settings
Erratic Results	A. Old or unhealthy cells used B. Adherent or non-adherent cells washed away C. Incorrect incubation time or temperature	A. Ensure cells are healthy prior to seeding and apoptosis induction B. Collect adherent and dislodged cells for analysis C. Incubate for recommended time and at recommended temperature

References

1. Chang, H.Y. and Yang, X. Proteases for cell suicide: Functions and regulation of caspases. *Microbiol. Mol. Biol. Rev.* **64(4)**, 821-846 (2000).
2. Chowdhury, I,, Tharakan, B., and Bhat, G.K. Caspases - An update. *Comp. Biochem. Physiol B Biochem. Mol. Biol.* **151(1)**, 10-27 (2008).
3. Chang, D.W., Ditsworth, D., Liu, H., *et al.* Oligomerization is a general mechanism for the activation of apoptosis initiator and inflammatory procaspases. *J. Biol. Chem.* **278(19)**, 16466-16469 (2003).
4. Cohen, G.M. Caspases: The executioners of apoptosis. *Biochem. J.* **326**, 1-16 (1997).
5. Howley, B. and Fearnhead, H.O. Caspases as therapeutic targets. *J. Cell. Mol. Med.* **12(5A),** 1502-1516 (2008).
6. Degterev, A., Boyce, M., and Yuan, J. A decade of caspases. *Oncogene* **22**, 8543-8567 (2003).

NOTES

Warranty and Limitation of Remedy

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