ApoSight™ Green Caspase 3/7 substrate

Catalog number: 13290
Unit size: 100 Tests

Component	Storage	Amount
ApoSight™ Green Caspase 3/7 Substrate	Freeze (< -15 °C), Minimize light exposure	100 Tests

OVERVIEW

ApoSight™ Green Caspase 3/7 substrate is the first fluorogenic probe for the direct fluorescence imaing of caspase activities in live cells. It consists of three moieties including a). a masked fluorophore, b). a caspase-selective peptide fragment (DEVD), and c). a cell-penetrating moiety. The cell-penetrating moiety carries the probe into live cells. Upon entering live cells, the caspase-selective peptide fragment is cleaved by a caspase to release the masked fluorophore. The intensity of recovered fluorescence is directly related to the activity of the caspase 3/7 to be measured. Compared to the existing caspase assays in live cells, ApoSight™ Green Caspase 3/7 substrate is much more robust, convenient, and accurate. ApoSight™ Green Caspase 3/7 substrate releases a fluorophore that has Ex/Em ~490/520 nm. It does not need a DNA interaction to be fluorescent as reported for NucView reagents. It does not inhibit caspase activity as reported for the FMK peptide probes. Although fluorescent FMK peptide inhibitors of caspases are widely used for detecting caspase activities in live cells, this technology has a few severe limitations: a). FMK caspase inhibitors have high cytotoxicity since FMK peptides bind covalently to active caspases; b). The irreversible covalent binding of FMK peptides to caspases inhibits caspase activities, causing false positive apoptosis; c). FMK assays have extremely high background, and require intensive washings, resulting in extremely low throughput; d). FMK peptides are not stable in aqueous solutions and must be used immediately. ApoSight™ Green Caspase 3/7 substrate can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds at a density of 5×10^4 to 2×10^5 cells/100 µL/well for 96-well plate
2. Add equal volume of ApoSight™ Caspase 3/7 Substrate working solution
3. Incubate in a 5% CO_2 incubator at 37 °C for 60 minutes
4. Wash the cells 1-2 time with HHBS
5. Analyze the cells with flow cytometer with 530/30 nm filter (FITC channel) or with fluorescence microscope with FITC filter set

KEY PARAMETERS

Flow cytometer

Excitation	488 nm laser
Emission	530/30 nm filter
Instrument specification(s)	FITC channel

Fluorescence microscope

Excitation	FITC filter set
Emission	FITC filter set
Recommended plate	Black wall/clear bottom

CELL PREPARATION

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

ApoSight™ Green Caspase 3/7 Substrate stock solution (200X)
Add 50 µL of DMSO into the vial of ApoSight™ Green Caspase 3/7 Substrate to make 200X ApoSight™ Green Caspase 3/7 Substrate stock solution.

Note Aliquot in single use aliquots to avoid repeated freeze-thaw cycle. Protect from light.

PREPARATION OF WORKING SOLUTION

ApoSight™ Green Caspase 3/7 Substrate working solution
Prepare ApoSight™ Green Caspase 3/7 Substrate working solution by mixing 5 µL of 200X ApoSight™ Green Caspase 3/7 Substrate stock solution with 1 mL of Hanks and 20 mM Hepes buffer (HHBS, Cat# 20011) or buffer of your choice and mix well.

Note 100 µL of ApoSight™ Green Caspase 3/7 Substrate working solution is enough for 10 tests in a 96-well plate format.

Note Prepare enough ApoSight™ Green Caspase 3/7 Substrate working solution right before the experiment, and use promptly.

SAMPLE EXPERIMENTAL PROTOCOL

Examples for inducing apoptosis in suspension culture
Treat Jurkat cells with 2 µg/mL camptothecin for 3 hours
Treat Jurkat cells with 1 µM staurosporine for 3-4 hours
Treat HL-60 cells with 4 µg/mL camptothecin for 4 hours
Treat HL-60 cells with 1 µM staurosporine for 4 hours.

Note Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction.

Sample protocol for Fluorescence Microscopy

1. Prepare cells with test compounds at a density of 5×10^4 to 2×10^5 cells/100 µL/well/96-well plate.
2. Add equal volume of Caspase 3/7 Substrate working solution to the cells (100 µL/well/96 well-plate).
3. Incubate in a 5% CO_2 incubator at 37 °C for 60 minutes.
4. Wash cells 1-2 times with HHBS or buffer of your choice.
5. Image with a fluorescence microscope using a FITC filter set.

Sample protocol for Flow Cytometry

1. Add 200 X ApoSight™ Green Caspase 3/7 Substrate stock solution into the cell solution at a 1:200 ratio, and incubate the cells in a 5% CO_2 incubator at 37 °C for 60 minutes.

   Note The cells can be concentrated up to ~5 X 10^6 cells/mL for ApoSight™ Green Caspase 3/7 Substrate labeling. The appropriate incubation time depends on the individual cell type and cell concentration used.

2. Monitor the fluorescence intensity with a flow cytometer using 530/30 nm filter (FITC channel).

   Note To increase the signal/background ratio, spin down the cells at ~200 g for 5 minutes, wash cells with 1 mL washing buffer such as HHBS or buffer of your choice ones, and resuspend the cells in the desired amount of washing.

EXAMPLE DATA ANALYSIS AND FIGURES

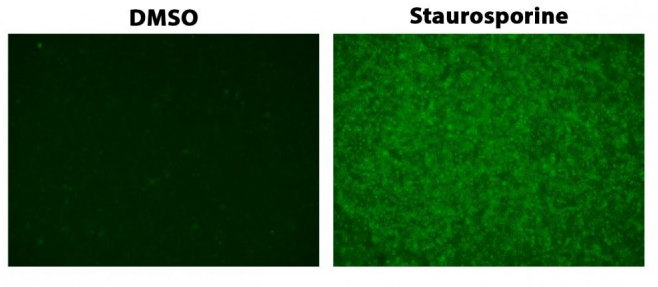

Figure 1. The detection of caspase 3/7 activity in Jurkat cells with ApoSight™ Green Caspase 3/7 substrate. Jurkat cells (200,000 cells/well/ 96-well plate) were treated with 1 µM Staurosporine or DMSO for 4 hours. Cells were incubated with Caspase 3/7 Substrate working solution at 37°C for 1 hour. Images were taken with a fluorescence microscope using a FITC filter set.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.