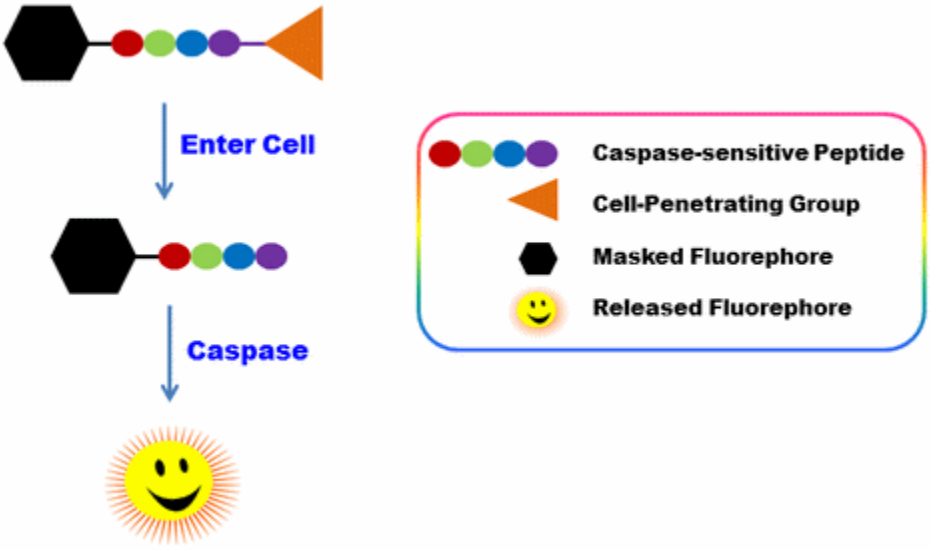
ApoBrite™ Caspases Substrates *Cell-Permeable*

Introduction

ApoBrite™ U470 and V570 are our recently developed cell-permeable fluorogenic caspase substrates, the first fluorogenic probes for the direct detection of caspase activities in live cells. ApoBrite U470 and V570 consist of three moieties including a). masked fluorophore, b). caspase-selective peptide fragment (DEVD), and c). 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 caspase to be measured. Compared to the existing caspase assays in live cells, ApoBrite™ U470 and V570 are much more robust, convenient and accurate. ApoBrite™ U470 and V570 release the fluorophores that have Ex/Em ~350/470 nm and 405/575 nm respectively. They do not need a DNA interaction to be fluorescent as reported for NucView reagents. They do 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 irreversibly 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 very low through put; d). FMK peptides are not stable in aqueous solutions, and have to be used immediately.



Chemical and Physical Properties

Catalog #	ApoBrite™ Caspases Substrate	Size	MW	Solvent	Excitation (nm)	Emission (nm)
20200	ApoBrite™ U470 caspase 3/7	100 tests	~1100	DMSO	312	475
20201	ApoBrite™ V570 caspase 3/7	100 tests	~1300	DMSO	405	575

Biological Applications

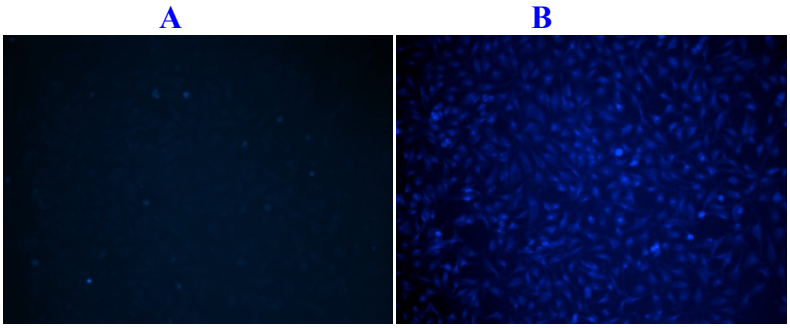
Cell permeable substrate for live cell caspases activity assays.
Recommended concentration: 5~20 μM in Hanks and 20 mM Hepes (HHBS) or other physiological buffer.

Assay Protocol with ApoBrite™ Caspases Substrates

This protocol only provides a guideline, and should be modified according to your specific needs. The ApoBrite™ substrates are 500X stock solutions in DMSO

1) Plate cells overnight in growth medium.
2) Make 1X ApoBrite™ caspase substrate working solution by adding 2 µL of 500X ApoBrite™ caspase substrates DMSO stock solution into 1 mL HHBS.
3) Remove growth medium, and wash once with HHBS.
4) Add 100 µL/well/96 well plate of 1X ApoBrite™ caspase substrate working solution, and incubate at 37 °C, 5% CO_2 incubator for 2 hours.
5) Remove ApoBrite™ caspase substrate working solution and wash once with HHBS.
6) Add growth medium back to the ApoBrite™ caspase substrate loaded cells, and treat the cells as desired (such as 1µM Staurosporine for 1 hour).
7) Analyze the cells with a proper fluorescence instrument (e.g., a fluorescence microscope, or flow cytometer).

Data Analysis:



The fluorescence microscope images of normal Hela cells (A) and apoptotic Hela cells (B). Hela cells were cultured in a 96-well plate, and washed twice with HHBS buffer. ApoBrite™ U470 caspase 3/7 dye loading solution was then added to the well. After incubation for 2 h at 37 °C, the cells were washed once with HHBS buffer and treated with staurosporine (1 µM) apoptosis inducer for 1 hr. The images were acquired using a fluorescence microscope equipped with DAPI filter set.

References

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