Cell Meter™ No-Wash Live Cell Caspase 3/7 Activity Assay Kit *Red Fluorescence*

Catalog number: 20260
Unit size: 200 Tests

Component	Storage	Amount
Component A: ApoBrite™ V570 Caspase 3/7 Substrate	Freeze (<-15 °C), Minimize light exposure	40 µL (500 X)
Component B: HBSS (Hanks and 20 mM Hepes Buffer)	Freeze (<-15 °C), Minimize light exposure	1 bottle (100 mL)
Component C: 500X 7-AAD	Freeze (<-15 °C), Minimize light exposure	1 vial (100 µL)

OVERVIEW

The activation of caspase 3/7 is important for the initiation of apoptosis. Our Cell Meter™ No-Wash Live Cell Caspase Activity Assay Kits are based on ApoBrite™ V600, our recently developed cell-permeable fluorogenic caspase substrate, the first fluorogenic probe for the direct detection of caspase activities in live cells. ApoBrite V600 consists 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™ V600 is much more robust, convenient and accurate. ApoBrite™ V600 releases a fluorophore that has a large Stokes shift, and can be well excited with violet laser that is installed most of new flow cytometers. 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 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.

AT A GLANCE

Protocol summary

1. Prepare cells
2. Remove the growth medium, wash cells
3. Incubate Caspase 3/7 Substrate working solution at 37 ºC for 2 hours
4. Wash cells and replace with growth medium
5. Induce apoptosis
6. Monitor fluorescence intensity (bottom read mode) at Ex/Em = 405/570 nm (Cutoff = 540 nm), fluorescence microscope with DAPI filter, or flow cytometer with Ex/Em = 405/470 nm filters

Important Thaw all the components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	DAPI channel
Emission:	DAPI channel
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	TRITC channel for 7-AAD staining
Instrument:	Flow cytometer
Excitation:	405 nm
Emission:	570 nm
Instrument specification(s):	FL3 channel for 7-AAD staining
Instrument:	Fluorescence microplate reader
Excitation:	405 nm
Emission:	570 nm
Cutoff:	540 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Bottom read mode

PREPARATION OF WORKING SOLUTION

Add 2 µL of 500X ApoBrite™ V570 Caspase 3/7 Substrate stock solution (Component A) into 1 mL HHBS (Component B) to make 1X ApoBrite™ V570 Caspase 3/7 Substrate working solution.

PREPARATION OF CELL SAMPLES

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

SAMPLE EXPERIMENTAL PROTOCOL

1. Culture cells to a density optimal for apoptosis induction according to your specific induction protocol, but not to exceed 2 x 10^6 cells/mL for suspension cells. For adherent cells, plate cells at 30,000 - 80,000 cells/well/96-well plate overnight.

2. Remove growth medium, and wash once with HHBS (Component B) or buffer of your choice.

3. Add 100 µL/well/96 well plate of 1X ApoBrite™ V570 Caspase 3/7 Substrate working solution, and incubate at 37 ºC, 5% CO2 incubator for 2 hours.

4. Remove 1X ApoBrite™ V570 Caspase 3/7 Substrate working solution and wash once with HHBS (Component B).

5. Add growth medium back to the ApoBrite™ V570 Caspase 3/7 loaded cells, and treat the cells as desired for apoptosis. Here are a few examples for inducing apoptosis in cell culture:

   a. Treating Jurkat cells with 2 µg/ml camptothecin for 3 hours.

   b. Treating Jurkat cells with 1 µM staurosporine for 3 hours. Hela cells for 1 hour.

   c. Treating HL-60 cells with 4 µg/ml camptothecin for 4 hours.

   d. Treating HL-60 cells with 1 µM staurosporine for 4 hours.

   e. Treating Hela cells with 1 µM staurosporine for 1 hour.

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

6. If desired, label the cells with a DNA stain (such as 7-AAD for dead cells).

7. Monitor the fluorescence intensity by fluorescence microscopy, flow cytometer, or fluorescence microplate reader at Ex/Em = 405/570 nm (for 7-AAD, Ex/Em = 535/650 nm).

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

For flow cytometry (suspension cells): Monitor the fluorescence intensity using Ex/Em = 405/570 nm filters (FL3 channel for 7-AAD staining). Gate on the cells of interest, excluding debris.

For fluorescence microscope: Observe cells under a fluorescence microscope using Ex/Em = 405/570 nm or DAPI channel (TRITC channel for 7-AAD staining).

For fluorescence microplate reader: Monitor the fluorescence intensity (bottom read mode) with a fluorescence plate reader at Ex/Em = 405/570 nm (Cutoff = 540 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

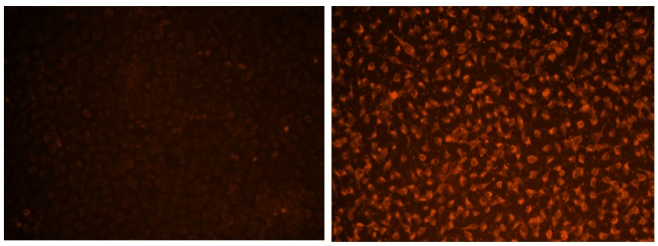

Figure 1.

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™ V570 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.

DISCLAIMER