Dyes and Stains

Caspase-3/7 Activity Plate Reader Assay Kit, Green

For detection of caspase-3/7 activity in cell lysates prepared from mammalian cells undergoing apoptosis

Catalog #100-0920

200 Tests

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Product Description

Caspases are a family of proteases that play a central role in cellular apoptosis through the cleavage of select proteins, and result in the disassembly of the cell (Thornberry & Lazebnik). This process is switched on by pro-apoptotic signals from death receptors or stimuli such as cytotoxic reagents, which activate the initiator caspases and lead to activation of the effector caspases—caspase-3 and caspase-7 (Thornberry & Lazebnik). Caspase-3 and caspase-7 have a proteolytic substrate selectivity for the peptide sequence Asp-Glu-Val-Asp (DEVD). This kit includes a fluorogenic caspase-3/7 substrate—Z-DEVD-Rh 110-DVED-Z—and an assay buffer. When added to apoptotic cells, Z-DEVD-Rh 110-DVED-Z is cleaved by activated caspase-3/7, generating a green fluorescent signal that can be detected by standard plate readers. Caspase assays are useful for quantifying caspase-3/7 activity in apoptotic cells and for screening caspase-3/7 inhibitors.

Excitation Wavelength: 500 nm
Emission Wavelength: 522 nm
Cutoff: 515 nm
Extinction Coefficient: 80,000 $cm^{-1}M^{-1}$
Format: Liquid

Product Information

The following components are sold as a complete kit (Catalog #100-0920) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
Caspase-3/7 Substrate (200X), Green*	300-0480	2 x 50 µL	Store at -20°C. Protect from prolonged exposure to light.	Product stable until expiry date (EXP) on label.
Assay Buffer	300-0481	20 mL	Store at -20°C.	Product stable until expiry date (EXP) on label.

*Please refer to the Safety Data Sheet (SDS) for hazard information. This product contains components dissolved in dimethyl sulfoxide (DMSO). DMSO is a strong solvent and skin penetrant, and can transport many substances through the skin. DMSO can also penetrate some protective glove materials including latex and silicone. Extra caution should be utilized when handling this product.

Directions for Use

Please read the entire protocol before proceeding. The following protocol is for staining cells in a black-wall/clear-bottom 96-well plate. If using other cultureware, adjust volumes accordingly.

Preparation of Caspase-3/7 Working Solution

1. Thaw Caspase-3/7 Substrate (200X), Green and Assay Buffer to room temperature (15 - 25°C).

   NOTE: If not used immediately, aliquot and store the substrate and buffer at -20°C. Do not exceed the expiry date as indicated on the label. After thawing aliquots, use immediately; do not re-freeze.
2. To prepare a caspase-3/7 working solution, add 50 µL of Caspase-3/7 Substrate (200X), Green to 10 mL of Assay Buffer. Mix thoroughly. Use the working solution immediately; do not store.

Cell Preparation

Adherent cells

1. Add cells to a black-wall/clear-bottom plate as follows:
   - 96-well plate: 2 x 10^4 cells in 90 µL of culture medium per well
   - 384-well plate: 5 x 10^3 cells in 20 µL of culture medium per well

   NOTE: The optimal plating density to induce apoptosis should be determined for different cell types.
2. Allow cells to adhere overnight before staining.

Non-adherent cells

1. Centrifuge cell suspension at 100 - 150 x *g* for 5 minutes. Remove supernatant.
2. Resuspend cells in a small volume of culture medium and perform a cell count with a hemocytometer.
3. Add cells to a black-wall/clear-bottom plate as follows:
   - 96-well plate: 8 x 10^4 cells in 90 µL of culture medium per well
   - 384-well plate: 2 x 10^4 cells in 20 µL of culture medium per well

   NOTE: The optimal plating density to induce apoptosis should be determined for different cell types.

   NOTE: Plates coated with poly-D lysine (e.g. Corning Catalog #356640 or 356697) may be used to promote cell attachment.
4. Centrifuge the plate at 100 - 150 x *g* for 2 minutes with the brake off.

Staining Cells

1. To induce apoptosis, prepare test compounds in phosphate-buffered saline (PBS) (e.g. Catalog #37350) or a buffer of choice and add to the cells as follows:
   - 96-well plate: 10 µL/well of test compounds at 10X concentration
   - 384-well plate: 5 µL/well of test compounds at 5X concentration
2. To prepare blank wells, add the same volume of compound and buffer into culture medium without cells.
3. Incubate the plate in a 37°C and 5% CO_2 incubator for an appropriate length of time to induce apoptosis.

   Example: Jurkat cells treated with camptothecin require 4 - 6 hours of incubation for apoptosis to occur.

   NOTE: The optimal cell density to induce apoptosis should be determined for different cell lines.
4. Add caspase-3/7 working solution to the plate as follows:
   - 96-well plate: 100 µL/well
   - 384-well plate: 25 µL/well
5. Incubate at room temperature for at least 1 hour; protect from light.

   OPTIONAL: To confirm inhibition of caspase-3/7-like activities, add pan-caspase inhibitor Z-VAD-FMK (e.g. Catalog #100-0534) to a final concentration of 50 µM to selected samples, then incubate at room temperature for 10 minutes prior to adding working solution. The concentration of Z-VAD-FMK should be optimized for different cell lines.
6. Centrifuge at 100 - 150 x *g* for 2 minutes with the brake off.

Imaging Stained Cells

Observe stained cells using a fluorescence microplate reader at Ex/Em = 490/525 nm (cutoff = 515 nm).

References

Thornberry NA & Lazebnik Y. (1998) Caspases: enemies within. Science 281(5381): 1312–6.