Dyes and Stains

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

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

Catalog #100-0922

100 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-ProRed™—and an assay buffer. When added to apoptotic cells, Z-DEVD-ProRed™ is cleaved by activated caspase-3/7, generating a red 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: 532 nm
Emission Wavelength: 619 nm
Cutoff: 610 nm
Format: Lyophilized powder

Product Information

The following components are sold as a complete kit (Catalog #100-0922) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
Caspase-3/7 Substrate (200X), Red	300-0484	1 vial	Store at -20°C. Protect from prolonged exposure to light.	Product stable until expiry date (EXP) on label.
Assay Buffer	300-0485	10 mL	Store at -20°C.	Product stable until expiry date (EXP) on label.

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. Warm the vial of Caspase-3/7 Substrate (200X), Red to room temperature (15 - 25°C) and centrifuge briefly before opening.
2. To prepare a caspase-3/7 stock solution, add 65 µL of dimethyl sulfoxide (DMSO) to the vial of Caspase-3/7 Substrate (200X), Red. Mix thoroughly.

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

   NOTE: If not used immediately, aliquot and store the buffer at -20°C. Do not exceed the expiry date as indicated on the label. After thawing aliquots, use immediately; do not re-freeze.

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 amount of culture medium and perform a cell count with a hemocytometer.
3. Add cells to a tissue culture-treated plate as follows:
   - 96-well plate (e.g. Catalog #27135): 8 x 10^4 to 2 x 10^5 cells in 90 µL of culture medium per well
   - 384-well plate: 2 x 10^4 to 5 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 = 540/620 nm (cutoff = 610 nm).

References

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