Dyes and Stains

Caspase-3/7 Activity Flow Cytometer Kit, Green

For detection of active caspase-3 in mammalian cells undergoing apoptosis

Catalog #100-0923 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 has substrate selectivity for the peptide sequence Asp-Glu-Val-Asp (DEVD). This kit includes a cell-permeable and non-toxic fluorogenic caspase-3/7 substrate—TF2-DEVD-FMK—as well as an assay buffer and propidium iodide. When added to apoptotic cells, TF2-DEVD-FMK irreversibly binds to activated caspase-3, resulting in the retention of the green fluorescent reagent within cells that can be detected by flow cytometry. Caspase-3/7 Activity Flow Cytometry Kit, Green is useful for screening caspase-3 inhibitors and quantifying apoptotic cells containing active caspase-3.

Excitation Wavelength: 503 nm
Emission Wavelength: 525 nm
Extinction Coefficient: 75,000 $cm^{-1}M^{-1}$
Format: Liquid

Product Information

The following components are sold as a complete kit (Catalog #100-0923) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
TF2-DEVD-FMK (500X)*	300-0486	100 µL	Store at -20°C. Protect from prolonged exposure to light.	Product stable until expiry date (EXP) on label.
Propidium Iodide (500X)*	300-0488	100 µL	Store at -20°C. Protect from prolonged exposure to light.	Product stable until expiry date (EXP) on label.
Assay Buffer	300-0487	50 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 2.5 x 10^5 to 5 x 10^5 cells in 0.5 mL of culture medium or suitable buffer per sample. If using other cell densities, adjust volumes accordingly.

1. Thaw TF2-DEVD-FMK (500X), Propidium Iodide (500X), and Assay Buffer at room temperature (15 - 25°C).
2. To induce apoptosis, treat cells with test compounds and incubate at 37°C and 5% CO_2 for an appropriate length of time.
   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.
3. Prepare positive and negative controls as follows:
   a. Positive control: camptothecin or staurosporine at 5X or 10X concentration
      NOTE: The optimal concentration should be determined for different cell lines.
   b. Negative control 1: untreated cells
   c. Negative control 2: untreated cells + 1 µL TF2-DEVD-FMK (500X)

4. Add TF2-DEVD-FMK (500X) to cells as follows:
   - Non-adherent cells: Add 1 µL of thawed TF2-DEVD-FMK (500X) to the cells.
   - Adherent cells: Gently lift the cells with 0.5 mM EDTA or suitable cell dissociation reagent and wash once with serum-containing medium before adding 1 µL of thawed TF2-DEVD-FMK (500X) to the cells.
5. Incubate at 37°C and 5% CO_2 for 1 - 4 hours.
   NOTE: The optimal incubation time should be determined for different cell lines and cell concentrations.
6. Centrifuge at 100 - 150 x *g* for 5 minutes. Remove supernatant and resuspend cells in Assay Buffer.
   NOTE: Centrifugation speed may need to be optimized to ensure apoptotic cells are sufficiently pelleted.
7. Repeat step 6 for a total of two washes with Assay Buffer to remove excess TF2-DEVD-FMK.
8. Resuspend the cells in 0.5 mL of Assay Buffer.
9. For a viability stain, add 1 µL of Propidium Iodide (500X) to each sample. Cells may be fixed at this stage before flow cytometry analysis.
10. Monitor the fluorescence intensity using a flow cytometer with 530/30 nm filter (FITC channel). Gate on cells of interest and exclude debris.

References

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