Caspase-3 Assay Kit For Live Cells

Cat. No. KITG-151 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	This kit uses a novel cell-permeable Caspase-3/7 green fluorescent substrate to detect Caspase-3/7 activity in live cells and can be used for real-time detection of apoptosis in live cells.
Description	Description The kit is designed for real-time detection of apoptosis in live cells. Unlike traditional caspase activity assay kits that usually require lysis of cells or tissue samples before colorimetric or fluorescent detection and therefore cannot be used for caspase activity detection in live cells, and unlike Western blotting that detects cleavage/activation of caspase itself but only reflects average enzyme activity in samples, the substrate in this kit does not inhibit the apoptotic process and allows real-time monitoring of apoptosis progression. Fluorescence-based caspase inhibitor detection technology such as FLICA can enter live cells to detect caspase activity, but its fluorescent probe is itself an irreversible caspase inhibitor and therefore cannot be used for real-time detection of caspase activity in live cells. Scientific Background Caspase-3 Substrate is a DEVD polypeptide coupled to a DNA green fluorescent dye. DEVD in the substrate is the recognition sequence of caspase 3/7 and carries a large number of negative charges. These negative charges repel the negative charges of DNA, so the DNA green fluorescent dye in the substrate cannot bind DNA and cannot be excited to produce green fluorescence. When the initially non-fluorescent substrate crosses the cell membrane into the cytoplasm, it is recognized and cleaved by Caspase-3/7 in apoptotic cells, releasing activated DNA green fluorescent dye molecules. When this DNA green fluorescent dye migrates into the nucleus and binds to DNA, it can be excited to produce bright green fluorescence. Kit Components Caspase-3 Substrate (1 mM): 20 µl for 40T; 100 µl for 200T. Ac-DEVD-CHO (10

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mM): 10 µl for 40T; 20 µl for 200T. Materials Required but Not Supplied Fresh cell culture medium or PBS or other appropriate solution for substrate treatment and dilution; trypsin or another appropriate method for adherent cell detachment for flow cytometry; black 96-well plate for adherent cells in microplate detection; culture medium or appropriate solution for flow cytometry and microplate detection; Hoechst 33342 live cell staining solution (100X) for optional nuclear counterstaining; 4% paraformaldehyde and 0.1% Triton X-100 permeabilization solution if fixation and permeabilization are desired; a non-reversible caspase inhibitor for cells that require it. Compatible Sample Types Live cells, including adherent cells and suspension cells. Features & Benefits 1. This kit can detect apoptosis in real time. Traditional caspase activity assay kits usually require lysed cells or tissue samples for colorimetric or fluorescent detection and cannot be used for caspase activity detection in live cells; Western blot detection of caspase cleavage/activation only reflects average caspase activity in samples. Fluorescence-labeled caspase inhibitor technology such as FLICA can enter live cells to detect caspase activity, but its fluorescent probe is itself an irreversible caspase inhibitor and cannot be used for real-time detection of caspase activity in live cells. The substrate in this kit does not inhibit the apoptotic process and can monitor apoptosis progression in real time. 2. The kit is dual-functional: it can detect Caspase-3/7 activity and can also show nuclear morphological changes during apoptosis. In many apoptotic cells, different cells may be in different stages and undergo different events during apoptosis; an important point in research is to track these events independently in real time to better understand their interrelationships. This kit can be used together with other fluorescent probes such as mitochondrial probes and other apoptosis probes to study the temporal and spatial relationships of these different events by fluorescence microscopy and flow cytometry. 3. The kit has strong downstream compatibility: after detection with this kit, cells can continue to be fixed with paraformaldehyde and used for subsequent immunostaining, including immunofluorescence and immunohistochemistry. 4. For a 96-well plate, when the detection system volume per

	well is 100 µl, 40 assays can be performed; when the detection system volume is 200 µl, only 20 assays can be performed. The actual number of assays may vary with the detection system and substrate concentration used.
Applications	Real-time detection and monitoring of apoptosis in live cells; Caspase-3/7 activity detection and visualization of nuclear morphological changes during apoptosis; use with other fluorescent probes such as mitochondrial probes or other apoptosis probes to study temporal and spatial relationships of different events by fluorescence microscopy or flow cytometry; subsequent immunostaining after detection, including immunofluorescence and immunohistochemistry, after paraformaldehyde fixation.
Notes	Product information is for reference only. For some cells, a non-reversible caspase inhibitor may be needed. Literature reports that apoptosis in a few cell types may not show detectable Caspase-3/7 activation. For research use only. Do not use for medical, clinical diagnosis or treatment, food or cosmetics. Do not store in ordinary residential areas. Keywords: Caspase-3; Caspase-3/7; live cells; apoptosis; green fluorescence; DEVD; Ac-DEVD-CHO Size: 40T/200T (using a 96-well plate with a total volume of 100 µl as an example). When the detection system volume in each well of a 96-well plate is 100 µl, 40 assays can be performed; when the detection system volume is 200 µl, only 20 assays can be performed. The actual number of assays may vary with the detection system and substrate concentration used. For safety and health, wear a lab coat and disposable gloves and mask during operation. Fluorescent dyes are somewhat quenchable; avoid prolonged observation under a fluorescence microscope.
Storage	-20°C, protected from light; valid for 1 year. Store at -20°C protected from light; shelf life is 1 year. Avoid repeated freeze-thaw cycles of kit components; if used multiple times, aliquot appropriately. Ac-DEVD-CHO may solidify and adhere to the bottom, wall or cap of the centrifuge tube at low temperature such as 4°C or ice bath; warm at 20-25°C in a water bath until completely dissolved before use.

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Reaction conditions	Fluorescence microscopy, flow cytometry and microplate reader detection of green fluorescence. For fluorescence microscopy and flow cytometry, use the green fluorescence filter/channel with maximum excitation/emission wavelength of 500/530 nm. For microplate reader, set excitation wavelength to 485 nm and emission wavelength to 515 nm; bottom reading is recommended for adherent cells. For optional nuclear staining with Hoechst 33342, the excitation/emission wavelength is 346/460 nm.
Usage	Preparation Optimize in advance: cell density, Caspase-3 Substrate concentration, inhibitor concentration and apoptosis induction conditions. A. Cell density should not be too low and is generally 50%-90%. B. The recommended working concentration of Caspase-3 Substrate is 5 μM; the optimal concentration can be optimized within 1-10 μM. The substrate can be added directly to cell culture medium, PBS or other appropriate solution to treat cells. For adherent cells, it is best to replace the culture medium before adding the substrate; after substrate incubation, whether to replace the culture medium can be decided as needed. C. The inhibitor Ac-DEVD-CHO can inhibit Caspase-3/7 activity. Because Ac-DEVD-CHO is a reversible inhibitor, it can be added to the culture medium in advance to pretreat cells, incubated at room temperature for 15-30 minutes, and then the substrate added; alternatively, the inhibitor can be added at the time of apoptosis induction, and the original inhibitor concentration can be supplemented when the medium is changed before substrate addition. The inhibitor concentration is generally 2-4 times the substrate concentration; for example, when using 5 μM substrate, use 10-20 μM inhibitor. Some cells require a non-reversible inhibitor. D. For apoptosis induction conditions of each cell line, refer to the literature. Assay Protocol Optimization: optimize cell density, Caspase-3 Substrate concentration, inhibitor concentration and apoptosis induction conditions in advance. Cell density should generally be 50%-90%. Recommended substrate concentration is 5 μM, with 1-10 μM as the range to optimize; for adherent cells, replace the medium before adding substrate and decide whether to replace medium after incubation as needed. Use Ac-DEVD-CH

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O at 2-4 times the substrate concentration; pretreat cells at room temperature for 15-30 minutes before substrate addition or supplement the same inhibitor concentration when changing medium before substrate addition if inhibitor was added at induction. For apoptosis induction conditions of each cell line, refer to the literature. Controls: set the following controls. A. Negative control: cells without apoptosis induction. B. Positive control: apoptosis induced without adding inhibitor Ac-DEVD-CHO. C. Positive control with inhibitor: apoptosis induced with Ac-DEVD-CHO added; this control is used to further confirm the detection effect of the kit. Fluorescence microscopy detection: A. Choose an appropriate method to induce apoptosis; untreated cells serve as the negative control; add a certain concentration of inhibitor to the inhibitor group. B. Replace the medium with fresh medium or PBS containing 5 μM Caspase-3 Substrate; for the Ac-DEVD-CHO inhibitor group, supplement the original concentration of inhibitor. C. Incubate at room temperature protected from light for 15-30 minutes; incubate longer if needed. D. Cells can be observed directly in medium containing substrate, or after medium replacement. Use a green fluorescence filter set with maximum excitation/emission wavelength of 500/530 nm. E. After counterstaining with Hoechst 33342 live cell staining solution (100X), nuclei show blue fluorescence. Flow cytometry detection: A. Choose an appropriate method to induce apoptosis; untreated cells serve as the negative control; add a certain concentration of inhibitor to the inhibitor group. B. For adherent cells, digest cells with trypsin or another method first. Resuspend cells in culture medium or buffer to a concentration of 10^6/ml. C. Transfer 200 μl to a flow cytometry tube, add 1 μl Caspase-3 Substrate, mix immediately to a final substrate concentration of 5 μM; optimize the appropriate substrate concentration for different cells. D. Incubate at room temperature protected from light for 15-30 minutes; incubate longer if needed. E. Add 300 μl culture medium or appropriate solution and analyze by flow cytometry. Detect the green fluorescence channel with maximum excitation/emission wavelength of 500/530 nm. Microplate reader detection: A. Grow adherent cells in a black 96-well plate; for suspension cells, adjust density to 10^6 cells/ml and add 0.2 ml cell suspension per well. B. Choose an appropriate method to induce apoptosis; untreat

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ed cells serve as the negative control; add a certain concentration of inhibitor to the inhibitor group. C. For adherent cells, replace the medium with fresh mediu m or PBS containing 5 μM Caspase-3 Substrate and supplement the original inhibito r concentration in the Ac-DEVD-CHO inhibitor group; for suspension cells, add substr ate or inhibitor directly. D. Incubate at room temperature protected from light for 15-30 minutes; incubate longer if needed. E. For suspension cells, gently shake to resuspen d cells. Set the microplate reader excitation wavelength to 485 nm and emission wave length to 515 nm; bottom reading is recommended for adherent cells. Changes in cell density may cause inaccurate readings. Nuclear staining: if necessary, stain nuclei wit h Hoechst 33342 dye; nuclei show blue fluorescence with excitation/emission wavele ngth of 346/460 nm. Assay Time Room temperature incubation protected from light fo r 15-30 minutes; longer incubation may be used if needed. Paraformaldehyde fixation, when used, is 10-15 minutes with 4% paraformaldehyde. Analysis Green fluorescenc e indicates Caspase-3/7 activity released by substrate cleavage and nuclear DNA bin ding in apoptotic cells; nuclear morphology can be assessed simultaneously. Use gre en fluorescence excitation/emission of 500/530 nm for microscopy/flow cytometry, 48 5/515 nm for microplate reader, and 346/460 nm for Hoechst 33342 nuclear counterst aining.

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