SANTA CRUZ BIOTECHNOLOGY, INC.

Caspase-3 Apoptosis Detection Kit: sc-4263 AK

BACKGROUND

The caspase-3 fluorometric protease assay utilizes a synthetic substrate, DEVD-AFC, to determine the activity of caspase-3. DEVD-AFC is composed of a synthethic tetrapeptide, DEVD, which corresponds to the upstream amino acid sequence of the caspase-3 cleavage site in PARP, and the fluorphore AFC (7-amino-4-trifluoromethyl coumarin). Cleavage of the substrate between D and AFC by caspase-3 or related caspases releases AFC. Free AFC emits a yellow-green fluorescence at 480-520 nm (peak at 505 nm) upon excitation at 400 nm. Apoptotic cells should emit high levels of free AFC, whereas non-apoptotic cells should emit low levels of free AFC.

PRODUCT

The Caspase-3 Apoptosis Detection Kit contains the following components. Sufficient reagent for 50 tests.

- 500 µl substrate peptide DEVD-AFC
- 25 ml Cell Lysis Buffer
- 25 ml 2x Reaction Buffer
- 1 ml 1 M DTT

STORAGE

Store AFC substrate and DTT solution at -20° C. Protect AFC substrate from light. Avoid repeated freeze/thaw cycles. Store Cell Lysis Buffer and Reaction Buffer at 4° C.

NOTE: This product is shipped on blue ice, however, individual components should be stored separately as indicated above.

PROCEDURE

- Induce apoptosis in cells by desired method. A control culture of non-induced cells should be incubated concurrently.
- Harvest cells at various time points after induction of apoptosis and analyze as described below.

For Analysis by Spectrofluorometry

- Prepare cell lysates at various time points after induction of apoptosis. For adherent cells, aspirate off medium, wash plate with PBS, aspirate off wash, and add Cell Lysis Buffer (provided) to plate at 1 ml per 2 x 10^6 cells. For suspension cells, count cells, pellet by centrifugation at 250xg for 10 minutes, wash pellet with PBS, and add Cell Lysis Buffer at 1 ml per 2 x 10^6 cells.
- Incubate cells in lysis buffer for 10 minutes on ice.
- For each reaction aliquot a constant volume of cell lysate. The amount of cell lysate needed to cleave the substrate will vary based on the extent of caspase-3 activation in the experimental system and should be titrated. The amount should be between 10-100 µl per reaction.
- Dilute 2x Reaction Buffer (provided) to 1x and add DTT to a final concentration of 10 mM prior to use. Prepare reaction mixtures by adding 1 ml of this buffer and 10 µl of DEVD-AFC substrate to each aliquot of cell lysate.
- Incubate the reaction mixtures for 1 hour at 37° C.
- Measure level of free AFC using a spectrofluorometer with an excitation wavelength of 400 nm and an emission wavelength range of 480-520 nm (peak at 505 nm). Compare level of emission of apoptotic cell lysates to that of non-apoptotic cell lysates.

For Analysis with Fluorescent Microplate Reader

- Prepare cell lysates as described above. Alternatively, culture adherent cells at a density of 2 x 10^4-1 x 10^5 in 96 well plates. For the latter procedure lysing the cells is optional.
- For each reaction aliquot a constant volume of cell lysate to each well of a 96 well plate. The amount of cell lysate needed to cleave the substrate will vary based on the extent of caspase-3 activation in the experimental system and should be titrated. The amount should be between 10-50 µl per reaction.
- Dilute 2x Reaction Buffer (provided) to 1x and add DTT to a final concentration of 10 mM prior to use. Add 200 µl of this buffer and 5 µl of DEVD-AFC substrate to each well containing cell lysate or cells in culture.
- Incubate the reaction mixtures for 1 hour at 37° C.
- Measure level of free AFC using a plate reader with a 400 nm excitation filter and a 505 nm emission filter. Compare level of emission of apoptotic cell lysates to that of non-apoptotic cell lysates.

For Analysis by Flow Cytometry

- Harvest cells by centrifugation at 800xg for 5 minutes. Discard supernatant. Gently resuspend ~2 x 10^6 cells in 1 ml PBS, centifuge and discard supernatant. Gently resuspend cells in 1 ml PBS.
- Prepare reaction mixtures by combining 100 µl of cell suspension, 400 µl PBS and 10 µl DEVD-AFC substrate.

SELECT PRODUCT CITATIONS

1. Bustamante, J., et al. 2002. Sequential NO production by mitochondria and endoplasmic reticulum during induced apoptosis. Nitric Oxide 6: 333-341.
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3. Bustamante, J., et al. 2004. Kinetic analysis of thapsigargin-induced thymocyte apoptosis. Free Radic. Biol. Med. 37: 1490-1498.
4. Hrzenjak, A., et al. 2006. Valproate inhibition of histone deacetylase 2 affects differentiation and decreases proliferation of endometrial stromal sarcoma cells. Mol. Cancer Ther. 5: 2203-2210.
5. Sharma, C.S., et al. 2008. Simulated microgravity activates apoptosis and NFκB in mice testis. Mol. Cell. Biochem. 313: 71-78.
6. Daubriac, J., et al. 2009. Malignant pleural mesothelioma cells resist anoikis as quiescent pluricellular aggregates. Cell Death Differ. 16: 1146-1155.
7. Periyakaruppan, A., et al. 2009. Uranium induces apoptosis in lung epithelial cells. Arch. Toxicol. 83: 595-600.

RESEARCH USE

For research use only, not for use in diagnostic procedures.