

Caspase-8 Apoptosis Detection Kit: sc-4267 AK

BACKGROUND

The caspase-8 fluorometric protease assay utilizes a synthetic substrate, IETD-AFC, to determine the activity of caspase-8. IETD-AFC is composed of a synthetic tetrapeptide, IETD, which corresponds to the upstream amino acid sequence of the caspase-8 cleavage site in caspase-3, and the fluorophore AFC (7-amino-4-trifluoromethyl coumarin). Cleavage of the substrate between D and AFC by caspase-8 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.

REFERENCES

1. Stennicke, H.R. and Salvesen, G.S. 1998. Properties of the caspases. *Biochim. Biophys. Acta* 1387: 17-31.
2. Takahashi, A., et al. 1996. Cleavage of lamin A by Mch2 a but not CPP32: multiple interleukin 1 β -converting enzyme-related proteases with distinct substrate recognition properties are active in apoptosis. *Proc. Natl. Acad. Sci. USA* 93: 8395-8400.

PRODUCT

The Caspase-8 Apoptosis Detection Kit contains the following components. Sufficient reagent for 50 tests.

- 500 μ l substrate peptide IETD-AFC
- 25 ml Cell Lysis Buffer
- 25 ml 2x Reaction Buffer
- 1 ml 1 M DTT

RESEARCH USE

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

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 2×10^6 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 2×10^6 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 μ l 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 μ l of IETD-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 2×10^4 - 1×10^5 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 μ l per reaction.
- Dilute 2x Reaction Buffer (provided) to 1x and add DTT to a final concentration of 10 mM prior to use. Add 200 μ l of this buffer and 5 μ l of IETD-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 $\sim 2 \times 10^6$ cells in 1 ml PBS, centrifuge and discard supernatant. Gently resuspend cells in 1 ml PBS.
- Prepare reaction mixtures by combining 100 μ l of cell suspension, 400 μ l PBS and 10 μ l IETD-AFC substrate.
- Incubate the reaction mixtures for 1 hour at 37° C.
- Measure free AFC by flow cytometry using UV excitation of 400 nm and having an emission wavelength range of 480-520 nm. Compare level of emission of apoptotic cells to that of non-apoptotic cells.

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.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.