

CaspSELECT™ Caspase-3 Immunoassay Kit (Fluorometric)

(Catalog #K163-100; 100 assays; Store kit at -20°C)

I. Introduction:

Activation of caspase-3 plays a key role in initiation of cellular events during apoptosis. The CaspSELECT™ Caspase-3 Immunoassay Kit provides an effective immunosorbent enzyme assay for specific, quantitative detection of caspase-3 activity in microtiter plates. The assay utilizes caspase-3 polyclonal antibody to capture activated caspase-3 from cell lysates. Caspase substrate DEVD-AFC is then added and is cleaved proportionally to the amount of activated caspase-3 in the cell lysate. The cleavage generates free AFC which can be analyzed fluorometrically (Ex./Em. = 400 nm/505 nm) using a fluorescence plate reader. The assay ensures absolute specific detection of caspase-3. Other known caspases and non-specific proteases are not detected.

II. Kit Contents:

Components	K163-100
	100 assays
Cell Lysis Buffer	25 ml
Coating Buffer	10 ml
Anti-Caspase-3 Antibody (20X)	0.5 ml
Blocking Buffer	15 ml
Incubation Buffer	100 ml
DTT (1 M)	400 µl
DEVD-AFC Substrate (1 mM)	500 µl
Positive Control (rh-Caspase-3)	10 units
Microtiter Plate	12 x 8 wells
Adhesive Plate Cover	2 sheets

III. Caspase-3 Immunoassay Procedure:

A. General Consideration:

- Read the entire protocol before beginning the procedure. After thawing, store Cell Lysis Buffer, Coating Buffer, Blocking Buffer, and Incubation Buffer at 4°C. All reagents are stable for up to 6 months.

B. Cell Lysates Preparation:

- Induce apoptosis in cells by desired method. Concurrently incubate a control culture *without* induction (5 x 10⁶ cells are needed for each assay).
- Wash cells with ice-cold PBS and centrifuge at 700 x g.
- Resuspend cells in 200 µl of chilled Cell Lysis Buffer and incubate cells on ice for 10 minutes.
- Centrifuge for 1 min at maximum speed in a microcentrifuge (10,000 x g).
- Save supernatant for direct assay or store at -20°C for up to 6 months.

C. Plate Coating Procedure:

- Make 1X Anti-Caspase-3 Coating Solution freshly by diluting the 20X antibody with Coating Buffer (e.g., for 10 tests, take 50 µl antibody solution and add 950 µl Coating Buffer).

- Add 100 µl of the 1X Anti-Caspase-3 Coating Solution to each well. Cover the plate tightly with an adhesive cover foil and incubate at 37°C for 1 hour or at 4°C overnight.
- Remove Coating solution. Block nonspecific binding by adding 150 µl of Blocking Buffer. Cover the plate tightly and incubate at RT for 30 minutes.
- Remove the solution. Wash 3 times with 150 µl Incubation Buffer.

D. Caspase-3 Assay Procedure:

- Add 100 µl cell lysate or 1 unit rh-caspase-3 prepared below (as positive control) to antibody-coated well. Cover the plate tightly and incubate at 37°C for 1 hour.
Note: The lyophilized rh-caspase-3 can be reconstituted to 10 µl PBS. Before use, dilute 1 µl to 100 µl Cell Lysis Buffer for each assay.
- Remove solutions. Wash 3 times with 150 µl Incubation Buffer.
- Add 94 µl Incubation Buffer, 5 µl DEVD-AFC and 1 µl DTT to each well.
- Cover the plate tightly and incubate for 2-4 hours at 37°C (Note: If activity is low, over night incubation at 37°C can be performed to increase sensitivity).
- Read samples at Ex. = 370-425 nm and Em. = 490-525 nm in a fluorescence microtiter plate reader.
Fold increase in caspase-3 activity can be determined by comparing these results with the level of the uninduced control.

RELATED PRODUCTS:

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- Caspase Assay Kits & Reagents
- Mitochondrial Apoptosis Kits & Reagents
- Nuclear Apoptosis Kits & Reagents
- Apoptosis Inducers and Apoptosis siRNA Vectors

Cell Fractionation System

- Mitochondria/Cytosol Fractionation Kit
- Nuclear/Cytosol Fractionation Kit
- Membrane Protein Extraction Kit
- Cytosol/Particulate Rapid Separation Kit
- Mammalian Cell Extraction Kit
- FractionPREP Fractionation System

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- Quick Cell Proliferation Assay Kit
- Senescence Detection Kit
- High Throughput Apoptosis/Cell Viability Assay Kits
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- DNA Damage Quantification Kit
- Glutathione & Nitric Oxide Fluorometric & Colorimetric Assay Kits

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- Akt & JNK Activity Assay Kits
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