Caspase-3 Immunoassay Kit

Catalog Number KA0739

100 assays

Version: 02

Intended for research use only

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Introduction

Background

Activation of caspase-3 plays a key role in initiation of cellular events during apoptosis. The 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.

General Information

Materials Supplied

List of component

Component	Amount
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	96 (8x12) wells
Adhesive Plate Cover	2 sheets

Storage Instruction

Store kit at -20°C.

Assay Protocol

Reagent Preparation

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.

Sample Preparation

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

Assay Procedure

- Plate Coating Procedure

1. 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).
2. 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.
3. Remove Coating solution. Block nonspecific binding by adding 150 µl of Blocking Buffer. Cover the plate tightly and incubate at RT for 30 minutes.
4. Remove the solution. Wash 3 times with 150 µl Incubation Buffer.

- Caspase-3 Assay

1. 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.
2. Remove solutions. Wash 3 times with 150 µl Incubation Buffer.
3. Add 94 µl Incubation Buffer, 5 µl DEVD-AFC and 1 µl DTT to each well.
4. 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*).
5. 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.