Store at -20°C

#9850

Caspase-7 Activity Assay Kit

☑1 Kit (40 assays)

rev02/04

Cell Signaling TECHNOLOGY®

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Description: The Caspase-7 Activity Assay includes all reagents required to measure Caspase-7 activity, including antibody, substrate, reaction buffer and cell extracts buffer. The assay system uses Caspase-7 antibody to selectively immunoprecipitate Caspase-7. Fluorometric substrate is added and is cleaved proportionally to the amount of activated Caspase-7, generating free fluorescent AFC. Free AFCs are determined fluorometrically at Imax = 505 nM. Compared to other commercially available Caspase-7 activity assays, this kit is designed to be more specific by using Caspase-7 antibody to selectively immunoprecipitate Caspase-7 from cell lysates.

Species Cross Reactivity: H

Kit Components:
Caspase-7 Antibody: Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH coupled) corresponding to human Caspase-7. Antibodies are purified by protein A and peptide affinity chromatography and supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C.

Fluorogenic Caspase-7 Substrate
Ac-Asp-Glu-Val-Asp-AFC

10X Cell Extracts Buffer: 1X concentration: 50mM Pipes/KOH (pH 6.5), 2mM EDTA, 0.1% Chaps, 5mM DTT, 20µg/ml Leupeptin, 10µg/ml pepstatin A, 10 µg/ml aproptinin

10X Reaction/Washing Buffer
1X concentration: 50mM HEPES (pH 7.5), 75mM NaCl, 5mM DTT, 0.1% Chaps 200X DTT (1 M DTT) 1 ml

Background: Caspase-7 (CMH-1, Mch3, ICE-LAP3) has been identified as a major contributor to the execution of apoptosis (2-4). Caspase-7 is an effector caspase (along with caspase-2 and -3), meaning that it cleaves essential cellular machinery rather than activating other caspases (5-8). Caspase-7 is cleaved by many enzymes, including caspases-3, -6, -8, -9 and granzyme B (1,4,5). Once activated, caspase-7 cleaves many of the same substrates as caspase-3, including poly (ADP-ribose) polymerase, or PARP (2,4).

Background References:

(1) Nuñez, G. et al. (1998) *Oncogene* 17, 3237–3245.

Products Included in Kit

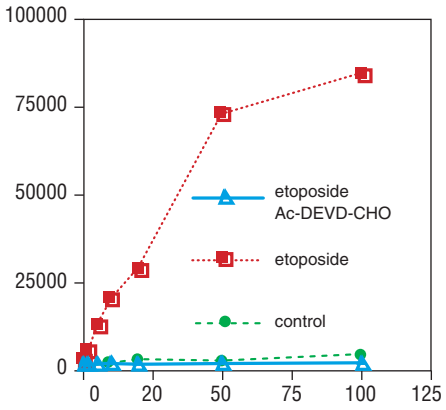
Products	Product #	Kit Quantity
Caspase-7 Antibody	[Antibody is unique to this kit.]	150 µl
Fluorogenic Caspase-7 Substrate		100 µl
Cell Extract Buffer (10X)	9852	5 ml
10X Reaction Buffer		15 ml
200X Dithiothreitol (1 M DTT)		1 ml

Caspase-7 Assay Kit Overview

Step 1: Selective IP of Caspase-7 using Caspase-7 Antibody
a) Add Caspase-7 Antibody
b) Add protein A sepharose beads*

Step 2: Incubate IP pellets in Reaction Buffer containing Caspase-7 Substrate (Ac-DEVD-AFC). Active Caspase-7 cleaves Ac-DEVD-AFC between DEVD and AFC, generating free fluorescent AFC.

Step 3: Measure free fluorescent AFC at excitation 400nM, emission 505nM.
*** reagent not included in kit**



Titration curve of Caspase-7 activity assay using varying amounts of Jurkat cells. Cells were either treated with etoposide for 6 hours or left untreated. Cell extracts were prepared for immunoprecipitation using Caspase-7 Antibody. Immunocomplexes were incubated with peptide substrate Ac-DEVD-AFC. Specific Caspase-7 activity was measured based on the fluorescent AFC group from the hydrolysis of Ac-DEVD-AFC by activated Caspase-7. Specific inhibition assay was performed by preincubating the immunocomplex with Ac-DEVD-CHO for 30 minutes before adding Ac-DEVD-AFC.

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(2) Fernandes-Alnemri, T. et al. (1995) *Cancer Res.* 55, 6045–6052.
(3) Duan, H. et al. (1996) *J. Biol. Chem.* 271, 1621–1625.
(4) Lippke, J.A. et al. (1996) *J. Biol. Chem.* 271, 1825–1828.
(5) Cohen, G.M. (1997) *Biochem. J.* 326, 1–16.
(6) Thornberry, N.A. et al. (1997) *J. Biol. Chem.* 272, 17907–17911.
(7) Chandler, J.M. et al. (1998) *J. Biol. Chem.* 273, 10815–10818.
(8) MacFarlane, M. et al. (1997) *J. Cell Biol.* 137, 469–479.

Companion Products:

Cleaved Caspase-7 (Asp198) Antibody #9491

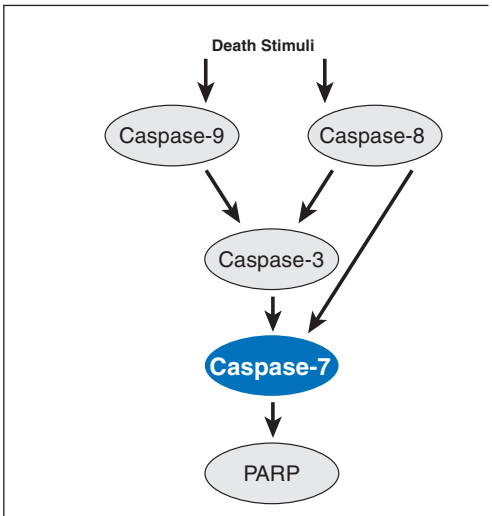
Caspase-7 Antibody #9492

Chaps Cell Exrtact Buffer (10x) #9852

Caspase-3 (3G2) mAb (human specific) #9668

Cleaved Caspase-3 (Asp175) (5A1) Rabbit Monoclonal Antibody #9664

Caspase-3 (10G1) Rabbit Monoclonal Antibody #9665



Recommended Antibody Dilutions

	Caspase-7 Antibody
Immunoprecipitation:	1:100

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **X**—*Xenopus* **Z**—zebra fish **All**—all species expected
Species enclosed in parentheses are predicted to react based on 100% sequence homology.

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Immunoprecipitation/Cleavage Reaction Protocols

A Solutions and Reagents

Note: Prepare solutions with Milli-Q or equivalently purified water.

A1 **Cell Extracts Buffer:** Immediately before use add DTT to final concentration of 5mM (1:200 dilution) and PMSF* to final concentration to 1mM.

A2 **Reaction/Washing Buffer:** immediately before use add DTT to final con centration of 5mM (1:200 dilution).

A3 ***Protein A Sepharose Beads:** (can be stored for 2 weeks at 4°C) Add 5 ml of 1X PBS to 1.5g of protein A sepharose beads, shake for 2 hours at 4°C. Spin down, wash pellet twice with PBS and resuspend beads in 1 volume of PBS.

*reagent not included in kit

B Preparing Cell Lysates

B1 Treat cells with regulator for desired time.

B2 To harvest cells under **nondenaturing conditions**, remove media and rinse cells once with ice-cold PBS.

B3 Pellet the cells and resuspend them in the appropriate volume of 1X ice-cold Cell Extracts Buffer plus 1mM PMSF.

B4 Freeze/thraw cells 3 times.

B5 Microcentrifuge at top speed for 10 minutes at 4°C; transfer the supernatant to a new tube. The supernatant is the cell lysate. If necessary, lysate can be stored at –80°C.

C Immunoprecipitation using Caspase 7 Antibodies:

C1 To 300 µl cell lysate, add Caspase-7 Antibody (1:100 dilution); incubate with gentle rocking for 2 hours.

C2 Add protein A sepharose beads (10-20 ul [50% beads]). Incubate with gentle rocking for 1–3 hours at 4°C.

C3 Microcentrifuge at 4,000 rpm for 30 seconds at 4°C. Remove unbound supernatant. Wash pellet twice with 1 ml of 1X Reaction Buffer. Keep on ice.

D Cleavage Reaction and Detection:

D1 Add 100 µL of Reaction Buffer containing a final concentration of 0.4mM Ac-DEVD-AFC (2 µL of the substrate stock solution) to the bead pellet.

D2 Incubate at 37°C overnight.

D3 The supernatant contains the free AFC. Measure AFC at excitation 400nM, emission 505nM.