

PathScan® Cleaved Caspase-3 (Asp175) Sandwich ELISA Kit

✓ 1 Kit
(96 assays)



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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Entrez-Gene ID #836
Swiss-Prot Acc. #P42574

Species Cross-Reactivity: H

Description: CST's PathScan® Cleaved Caspase-3 (Asp175) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of cleaved caspase-3 protein. A Cleaved Caspase-3 (Asp175) Rabbit mAb has been coated onto the microwells. After incubation with cell lysates, the cleaved caspase-3 protein is captured by the coated antibody. Following extensive washing, a Biotinylated Caspase-3 Rabbit Antibody is added to detect the captured cleaved caspase-3 protein. HRP-linked streptavidin is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of optical density for this developed color is proportional to the quantity of cleaved caspase-3 protein.

Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: CST's PathScan® Cleaved Caspase-3 (Asp175) Sandwich ELISA Kit detects endogenous levels of cleaved caspase-3 protein in human cell lines. Using this ELISA kit #7190, a significant induction of cleaved caspase-3 at aspartic acid 175 can be seen in staurosporine-treated HeLa cells, while the level of total caspase-3, analyzed by western blot using Caspase-3 Antibody #9662, remains unchanged (Figure 1). Jurkat cells treated with etoposide show similar results (data not shown). This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

Background: Caspase-3 (CPP-32, Apoptain, Yama, SCA-1) is a critical executioner of apoptosis, as it is either partially or totally responsible for the proteolytic cleavage of many key proteins such as the nuclear enzyme poly(ADP-ribose) polymerase (PARP) (1). Activation of caspase-3 requires proteolytic processing of its inactive zymogen into activated p17 and p12 fragments. Cleavage of caspase-3 requires aspartic acid at the P1 position (2).

Background References:

- (1) Fernandes-Alnemri, T. et al. (1994) *J. Biol. Chem.* 269, 30761-30764.
- (2) Nicholson, D. W. et al. (1995) *Nature* 376, 37-43.
- (3) Steiner, P. et al. (2007) *Clin Cancer Res* 13, 1540-51.

Products Included	Volume	Solution Color
Cleaved-Caspase 3 (Asp175) Rabbit mAb coated Microwells*	96 tests	
Biotinylated Caspase-3 Rabbit Detection Antibody	11 ml	green
HRP-Linked Streptavidin	11 ml	red
TMB Substrate	11 ml	colorless
STOP Solution	11 ml	colorless
Sealing Tape	2 sheets	
20X Wash Buffer	25 ml	colorless
Sample Diluent	25 ml	blue
10X Cell Lysis Buffer #9803**	15 ml	yellowish

* 12 8-well modules -Each module is designed to break apart for 8 tests.

**Kit should be stored at 4°C with the exception of 10X Cell Lysis Buffer, which is stored at -20°C (packaged separately).

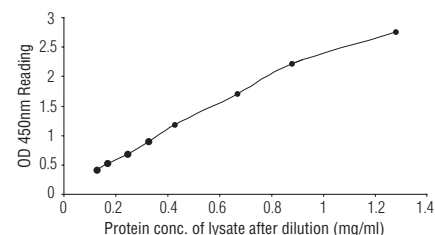
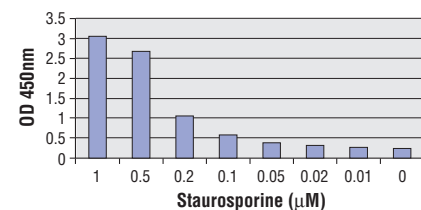


Figure 2: Linear relationship between protein concentration of staurosporine-treated HeLa cell lysates and kit assay optical density readings. HeLa cells (90% confluence) were treated with staurosporine (1 µM), and lysed after incubation at 37°C for 3 hours.

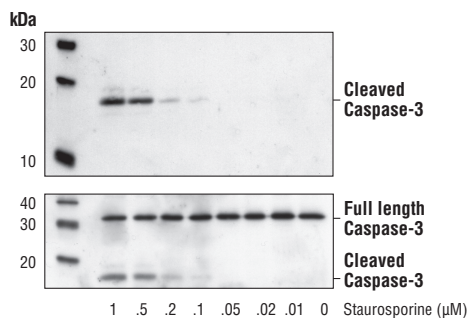


Figure 1: Treatment of HeLa cells with staurosporine stimulates cleavage of caspase-3 protein, detected by PathScan® Cleaved Caspase-3 (Asp175) Sandwich ELISA Kit #7190 (top figure), but does not affect the level of total caspase-3 protein detected by western blot (bottom figure), using Cleaved Caspase-3 (Asp175) Antibody #9661 (upper panel) or Caspase-3 Antibody #9662 (lower panel).

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide

Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine

Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—horse

All—all species expected

Species enclosed in parentheses are predicted to react based on 100% homology.

Sandwich ELISA Protocol

A Reagent Preparation

1. Bring all microwell strips to room temperature before use.
2. Prepare 1X Wash Buffer by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in Milli-Q or equivalently purified water.
3. **1X Cell Lysis Buffer from CST #9803:** 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM ethylene diamine tetraacetate (EDTA), 1 mM ethylene glycol-bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β -glycerophosphate, 1 mM Na_3VO_4 , 1 $\mu\text{g}/\text{ml}$ leupeptin. This buffer can be stored at 4°C for short-term use (1–2 weeks).

B Preparing Cell Lysates

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM phenyl-methylsulfonyl fluoride (PMSF) to each plate (10 cm in diameter) and incubate the plate on ice for 5 minutes.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 minutes at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

C Test Procedure

1. After the microwell strips have reached room temperature, break off the required number of microwells. Place the microwells in the strip holder. Unused microwells must be resealed and stored at 4°C immediately.
2. Add 100 μl of Sample Diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color) to a microcentrifuge tube. Transfer 100 μl of cell lysate into the tube and vortex for a few seconds. Generally, sample applied to the well can be diluted 1:1 when the suggested cell lysis buffer is used for cell extraction. Individual data sheets for each kit provide information regarding an appropriate dilution factor for lysates and kit assay results. However, dilution factors need to be titrated when specific cell lysates are used.

3. Add 100 μl of each diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hours at 37°C. Alternatively, the plate can be incubated overnight at 4°C, which gives the best detection of target protein.
4. Gently remove the tape and wash wells:
 - a. Discard plate contents into a receptacle.
 - b. Wash 4 times with 1X Wash Buffer, 200 μl each time for each well.
 - c. For each wash, strike plates on fresh towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
 - d. Clean the underside of all wells with a lint-free tissue.
5. Add 100 μl of Detection Antibody (green color) to each well. Seal with tape and incubate the plate for 1 hour at 37°C.
6. Repeat wash procedure as in Step 4.
7. Add 100 μl of HRP-linked secondary antibody (red color) to each well. Seal with tape and incubate the plate for 30 minutes at 37°C.
8. Repeat wash procedure as in Step 4.
9. Add 100 μl of TMB Substrate to each well. Seal with tape and incubate the plate for 10 minutes at 37°C or 30 minutes at 25°C.
10. Add 100 μl of STOP Solution to each well. Shake gently for a few seconds.

NOTE: Initial color of positive reaction is blue, which changes to yellow upon addition of STOP Solution.

11. Read results.
 - a. Visual Determination — Read within 30 minutes after adding STOP Solution.
 - b. Spectrophotometric Determination — Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 minutes after adding STOP Solution.