

Product datasheet

Human Cleaved Caspase-3 (Asp175) ELISA Kit ab220655

Recombinant SimpleStep ELISA®

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Overview

Product name Human Cleaved Caspase-3 (Asp175) ELISA Kit

Detection method Colorimetric

Precision

Intra-assay

Sample	n	Mean	SD	CV%
Cell extract	3			4.2%

Inter-assay

Sample	n	Mean	SD	CV%
Cell extract	5			5.2%

Sample type Cell culture extracts, Tissue Extracts

Assay type Sandwich (quantitative)

Sensitivity 2.1 pg/ml

Range 15.63 pg/ml - 1000 pg/ml

Recovery

Sample specific recovery

Sample type	Average %	Range
Cell culture extracts	108	105% - 115%

Assay time 1h 30m

Assay duration One step assay

Species reactivity **Reacts with:** Human

Product overview

Human Cleaved Caspase-3 (Asp175) ELISA Kit (ab220655) is a single-wash 90 min sandwich ELISA designed for the quantitative measurement of Cleaved Caspase-3 (Asp175) protein in cell culture extracts and tissue extracts. It uses our proprietary SimpleStep ELISA® technology.

Quantitate Human Cleaved Caspase-3 (Asp175) with 2.1 pg/ml sensitivity.

SimpleStep ELISA® technology employs capture antibodies conjugated to an affinity tag that is recognized by the monoclonal antibody used to coat our SimpleStep ELISA® plates. This approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. See the SimpleStep ELISA® protocol summary in the image section for further details. Our SimpleStep ELISA® technology provides several benefits:

- Single-wash protocol reduces assay time to 90 minutes or less
- High sensitivity, specificity and reproducibility from superior antibodies
- Fully validated in biological samples
- 96-wells plate breakable into 12 x 8 wells strips

A 384-well SimpleStep ELISA® microplate ([ab203359](#)) is available to use as an alternative to the 96-well microplate provided with SimpleStep ELISA® kits.

Notes

Caspase-3 is a cytoplasmic cysteine protease involved in the activation cascade of caspases responsible for execution of apoptosis. At the onset of apoptosis caspase-3 cleaves and activates caspase-6, -7 and -9. It also cleaves poly (ADP-ribose) polymerase (PARP). Caspase-3 cleaves and activates sterol regulatory element binding proteins (SREBPs). Caspase-3 is involved in the cleavage of huntingtin. Caspase-3 is expressed in an inactive pro-form (pro caspase-3). In apoptosis, the pro caspase-3 is activated by proteolytic cleavages at Asp28-Ser29 and Asp175-Ser176 bonds catalyzed by granzyme B, caspase-6, caspase-8, caspase-9 and caspase-10, generating two subunits p17 and p12 that are assembled into heterotetrameric active enzyme. Additional processing of the propeptides is likely due to the autocatalytic activity of the activated protease. The pro-form and the active form are useful biomarkers of apoptosis.

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances.

It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

Platform

Microplate (12 x 8 well strips)

Properties

Storage instructions Store at +4°C. Please refer to protocols.

Components	1 x 96 tests
10X Human Cleaved Caspase-3 (Asp175) Capture Antibody	1 x 600µl
10X Human Cleaved Caspase-3 (Asp175) Detector Antibody	1 x 600µl
10X Wash Buffer PT (ab206977)	1 x 20ml
50X Cell Extraction Enhancer Solution (ab193971)	1 x 1ml
5X Cell Extraction Buffer PTR (ab193970)	1 x 10ml

Components	1 x 96 tests
Antibody Diluent CPI - HAMA Blocker (ab193969)	1 x 6ml
Human Cleaved Caspase-3 (Asp175) Lyophilized Recombinant Protein	2 vials
Plate Seals	1 unit
Sample Diluent NS (ab193972)	1 x 12ml
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 x 96 tests
Stop Solution	1 x 12ml
TMB Development Solution	1 x 12ml

Function

Involved in the activation cascade of caspases responsible for apoptosis execution. At the onset of apoptosis it proteolytically cleaves poly(ADP-ribose) polymerase (PARP) at a '216-Asp-Gly-217' bond. Cleaves and activates sterol regulatory element binding proteins (SREBPs) between the basic helix-loop-helix leucine zipper domain and the membrane attachment domain. Cleaves and activates caspase-6, -7 and -9. Involved in the cleavage of huntingtin. Triggers cell adhesion in sympathetic neurons through RET cleavage.

Tissue specificity

Highly expressed in lung, spleen, heart, liver and kidney. Moderate levels in brain and skeletal muscle, and low in testis. Also found in many cell lines, highest expression in cells of the immune system.

Sequence similarities

Belongs to the peptidase C14A family.

Post-translational modifications

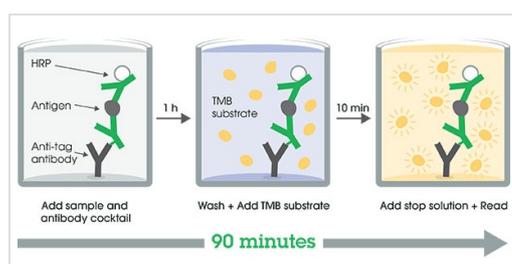
Cleavage by granzyme B, caspase-6, caspase-8 and caspase-10 generates the two active subunits. Additional processing of the propeptides is likely due to the autocatalytic activity of the activated protease. Active heterodimers between the small subunit of caspase-7 protease and the large subunit of caspase-3 also occur and vice versa.

S-nitrosylated on its catalytic site cysteine in unstimulated human cell lines and denitrosylated upon activation of the Fas apoptotic pathway, associated with an increase in intracellular caspase activity. Fas therefore activates caspase-3 not only by inducing the cleavage of the caspase zymogen to its active subunits, but also by stimulating the denitrosylation of its active site thiol.

Cellular localization

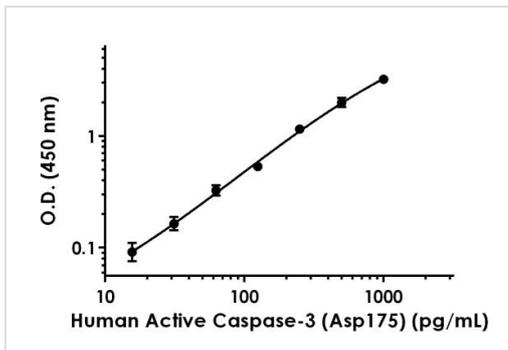
Cytoplasm.

Images



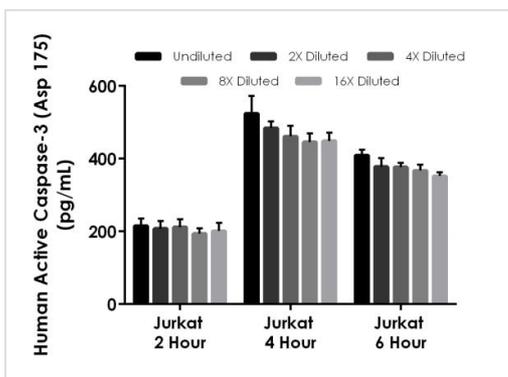
SimpleStep ELISA technology allows the formation of the antibody-antigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.

Other - Human Cleaved Caspase-3 (Asp175) ELISA Kit (ab220655)



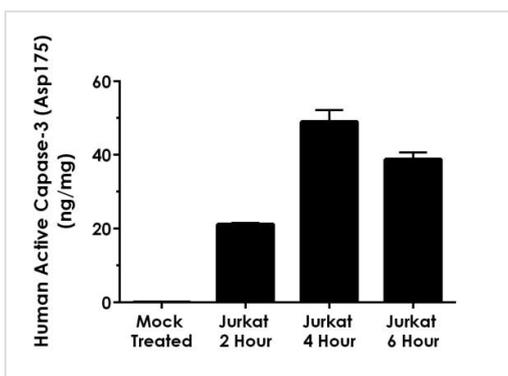
Example of human Active Caspase-3 (Asp175) standard curve in 1X Cell Extraction Buffer PTR.

Background-subtracted data values (mean +/- SD) are graphed.



Interpolated concentrations of native Active Caspase-3 (Asp175) in staurosporine treated Jurkat cell extract samples collected at different time points.

Jurkat cells were cultured in the presence of 1 μ M staurosporine and collected at time points of 2 hours, 4 hours and 6 hours based on a 10 μ g/mL extract load. The concentrations of Active Caspase-3 (Asp175) were measured in duplicate and interpolated from the Active Caspase-3 (Asp175) standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Active Caspase-3 (Asp175) concentration was determined to be 206.0 pg/mL in staurosporine treated Jurkat (2 hour), 472.9 pg/mL in staurosporine treated Jurkat (4 hour) and 376.7 pg/mL in staurosporine treated Jurkat (6 hour) cell extracts.



Comparison of staurosporine treated Jurkat cell extracts collected at different time points during treatment.

Jurkat cells were cultured in the presence of 1 μ g/mL staurosporine and collected at time points of 2 hours, 4 hours and 6 hours, or in the presence of staurosporine solvent (mock) collected at 4 hours. The concentration of Active Caspase-3 (Asp175) was measured in three different dilutions of the cell extract samples in duplicate and interpolated from the Active Caspase-3 (Asp175) standard curve. The interpolated dilution factor corrected values are plotted in ng of Active Caspase-3 (Asp175) per mg of extract (mean +/- SD, n=3). The mean Active Caspase-3 (Asp175) concentration was determined to be not detectable in Jurkat (mock treated), 21.16 ng/mg in Jurkat (2 hour), 49.00 ng/mg in Jurkat (4 hour) and 38.79 ng/mg in Jurkat (6 hour) cell extracts.

Powered by
recombinant antibodies



Research with confidence
Consistent and reproducible results



Long-term and scalable supply
Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

Sandwich ELISA - Human Cleaved Caspase-3
(Asp175) ELISA Kit (ab220655)

To learn more about the advantages of recombinant antibodies see [here](#).

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