abcam

Product datasheet

Anti-Caspase-7 antibody [E22] ab32522

KO VALIDATED RabMAb

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Overview

Product name	Anti-Caspase-7 antibody [E22]
Description	Rabbit monoclonal [E22] to Caspase-7
Host species	Rabbit
Specificity	The antibody should recognize both pro-form and p20 cleaved-form. The antibody does not cross-react with other Caspase family members.
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF, IP, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Jurkat and HeLa whole cell lysate (**ab150035**). IHC-P: Human skin cancer tissue. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa cells. IP: Jurkat cell lysates
General notes	Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide

	Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	E22
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32522 in the following tested applications.

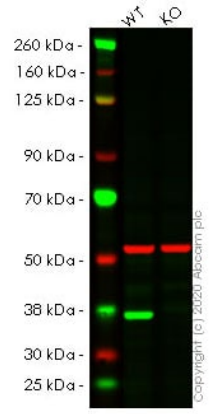
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★☆☆ (1)	1/1000. Detects a band of approximately 34 kDa (predicted molecular weight: 34 kDa).
IHC-P		1/50 - 1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		1/100.
IP	★★★☆☆ (1)	1/20.
Flow Cyt (Intra)		1/250.

Target

Function	Involved in the activation cascade of caspases responsible for apoptosis execution. Cleaves and activates sterol regulatory element binding proteins (SREBPs). Proteolytically cleaves poly(ADP-ribose) polymerase (PARP) at a '216-Asp- -Gly-217' bond. Overexpression promotes programmed cell death.
Tissue specificity	Highly expressed in lung, skeletal muscle, liver, kidney, spleen and heart, and moderately in testis. No expression in the brain.
Sequence similarities	Belongs to the peptidase C14A family.
Post-translational modifications	Cleavages by granzyme B or caspase-10 generate the two active subunits. Propeptide domains can also be cleaved efficiently by caspase-3. Active heterodimers between the small subunit of caspase-7 and the large subunit of caspase-3, and vice versa, also occur.
Cellular localization	Cytoplasm.

Images



Western blot - Anti-Caspase-7 antibody [E22] (ab32522)

All lanes : Anti-Caspase-7 antibody [E22] (ab32522) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : CASP7 knockout HeLa cell lysate

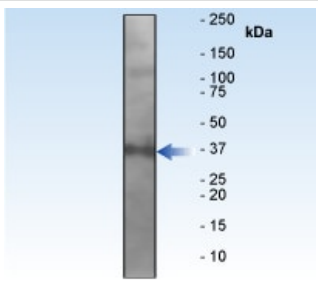
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 34 kDa
Observed band size: 38 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab32522 observed at 38 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

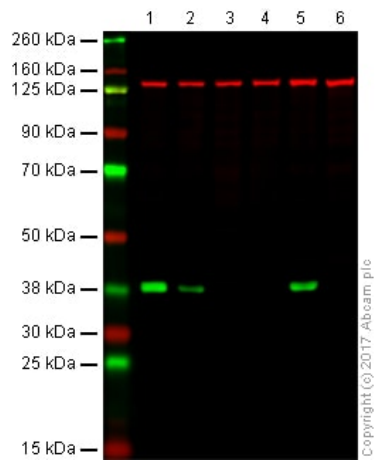
ab32522 was shown to react with pro Caspase-7 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab265777** (knockout cell lysate **ab257380**) was used. Wild-type HeLa and CASP7 knockout HeLa cell lysates were subjected to SDS-PAGE. ab32522 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Caspase-7 antibody [E22] (ab32522)

Anti-Caspase-7 antibody [E22] (ab32522) at 1/1000 dilution + Jurkat cell lysate

Predicted band size: 34 kDa
Observed band size: 34 kDa

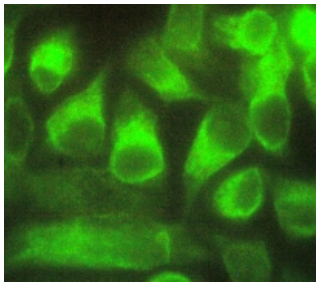


Western blot - Anti-Caspase-7 antibody [E22] (ab32522)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: Wild type HAP1 + Staurosporine **ab120056** whole cell lysate (20 µg)
Lane 3: CASP7 knockout HAP1 whole cell lysate (20 µg)
Lane 4: CASP7 + Staurosporine knockout HAP1 whole cell lysate (20 µg)
Lane 5: HeLa whole cell lysate (20 µg)
Lane 6: HeLa + Staurosporine whole cell lysate (20 µg)

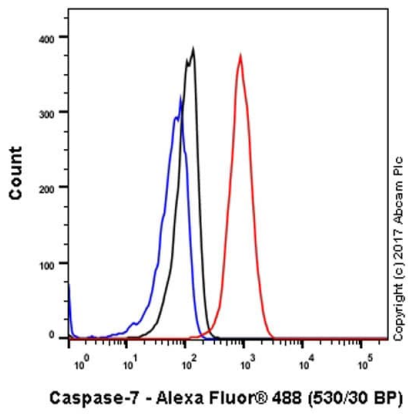
Lanes 1 - 6: Merged signal (red and green). Green - ab32522 observed at 38 kDa. Red - loading control, **ab18058**, observed at 130 kDa.

ab32522 was shown to specifically react with HAP1 + Staurosporine when HAP1 + Staurosporine knockout samples were used. Wild-type and HAP1 + Staurosporine knockout samples were subjected to SDS-PAGE. Ab32522 and **ab18058** (Mouse anti Vinculin loading control) were incubated overnight at 4°C at 1000 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



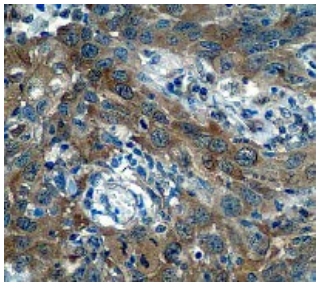
Immunocytochemistry/ Immunofluorescence - Anti-Caspase-7 antibody [E22] (ab32522)

Immunofluorescent staining of HeLa cells using ab32522 at 1:100 dilution.



Flow Cytometry (Intracellular) - Anti-Caspase-7 antibody [E22] (ab32522)

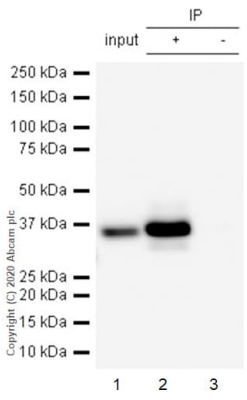
Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling Caspase-7 (red) with ab32522 at a 1/250 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A goat anti-rabbit IgG (Alexa Fluorr® 488) (**ab150077**) was used as the secondary antibody at a 1/2000 dilution. Black - Rabbit monoclonal IgG (**ab172730**). Blue (unlabeled control) - Cells without incubation with the primary and secondary antibodies.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caspase-7 antibody [E22] (ab32522)

Immunohistochemical analysis of paraffin embedded human skin cancer tissue using ab32522 at 1:50 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-Caspase-7 antibody [E22] (ab32522)

Purified ab32522 at 1/20 dilution (1µg) immunoprecipitating Caspase-7 in Jurkat whole cell lysate.
Lane 1 (input): Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate 10µg
Lane 2 (+): ab32522 + Jurkat whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32522 in Jurkat whole cell lysate.
VeriBlot for IP Detection Reagent (HRP) (**ab131366**) (1/1000 dilution) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: 34 kDa

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