abcam

Product datasheet

Anti-Caspase-8 antibody [E7] ab32397

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Caspase-8 antibody [E7]
Description	Rabbit monoclonal [E7] to Caspase-8
Host species	Rabbit
Specificity	The antibody should recognize both pro-form (55kDa) and p18 cleaved-form of Caspase-8.
Tested applications	**Suitable for:** WB **Unsuitable for:** Flow Cyt or ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human Caspase-8 aa 200-300 (N terminal). The exact sequence is proprietary. Database link: **Q14790**
Positive control	WB: Wild-type/Wild-type treated with Staurosporin HAP1, Jurkat, SH-SY5Y, IM-9, and HeLa (**ab150035**) whole cell lysates.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. 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. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	E7
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32397 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	★★★☆☆ **(2)**	1/500 - 1/1000. Detects a band of approximately 55 kDa (predicted molecular weight: 55 kDa). **For unpurified use at 1/500.**

Application notes Is unsuitable for Flow Cyt or ICC/IF.

Target

Function Most upstream protease of the activation cascade of caspases responsible for the TNFRSF6/FAS mediated and TNFRSF1A induced cell death. Binding to the adapter molecule FADD recruits it to either receptor. The resulting aggregate called death-inducing signaling complex (DISC) performs CASP8 proteolytic activation. The active dimeric enzyme is then liberated from the DISC and free to activate downstream apoptotic proteases. Proteolytic fragments of the N-terminal propeptide (termed CAP3, CAP5 and CAP6) are likely retained in the DISC. Cleaves and activates CASP3, CASP4, CASP6, CASP7, CASP9 and CASP10. May participate in the GZMB apoptotic pathways. Cleaves ADPRT. Hydrolyzes the small-molecule substrate, Ac-Asp-Glu-Val-Asp-
-AMC. Likely target for the cowpox virus CRMA death inhibitory protein. Isoform 5, isoform 6, isoform 7 and isoform 8 lack the catalytic site and may interfere with the pro-apoptotic activity of the complex.

Tissue specificity Isoform 1, isoform 5 and isoform 7 are expressed in a wide variety of tissues. Highest expression in peripheral blood leukocytes, spleen, thymus and liver. Barely detectable in brain, testis and skeletal muscle.

Involvement in disease Defects in CASP8 are the cause of caspase-8 deficiency (CASP8D) [MIM:607271]. CASP8D is a disorder resembling autoimmune lymphoproliferative syndrome (ALPS). It is characterized by lymphadenopathy, splenomegaly, and defective CD95-induced apoptosis of peripheral blood lymphocytes (PBLs). It leads to defects in activation of T-lymphocytes, B-lymphocytes, and natural killer cells leading to immunodeficiency characterized by recurrent sinopulmonary and herpes simplex virus infections and poor responses to immunization.

Sequence similarities Belongs to the peptidase C14A family.
Contains 2 DED (death effector) domains.

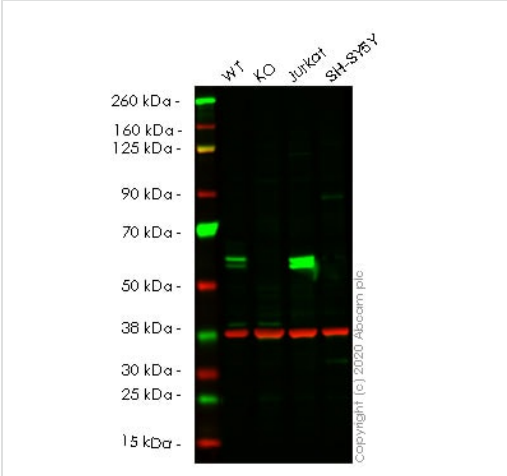
Domain Isoform 9 contains a N-terminal extension that is required for interaction with the BCAP31 complex.

Post-translational modifications Generation of the subunits requires association with the death-inducing signaling complex (DISC), whereas additional processing is likely due to the autocatalytic activity of the activated protease.

GZMB and CASP10 can be involved in these processing events.
Phosphorylated upon DNA damage, probably by ATM or ATR.

Cellular localization Cytoplasm.

Images



Western blot - Anti-Caspase-8 antibody [E7] (ab32397)

All lanes : Anti-Caspase-8 antibody [E7] (ab32397) at 1/500 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : CASP8 knockout HeLa cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : SH-SY5Y cell lysate

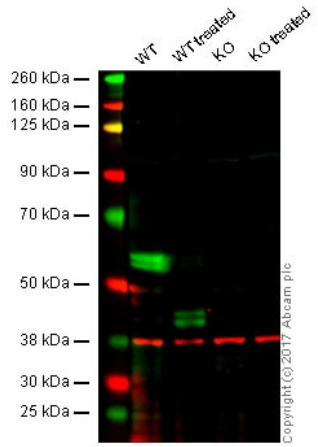
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 55 kDa
Observed band size: 55 kDa

Lanes 1- 4: Merged signal (red and green). Green - ab32397 observed at 55 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab32397 was shown to react with Caspase-8 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab264958** (knockout cell lysate **ab256857**) was used. Wild-type HeLa and CASP8 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab32397 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 500 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-8 antibody [E7] (ab32397)

All lanes : Anti-Caspase-8 antibody [E7] (ab32397) at 1/500 dilution

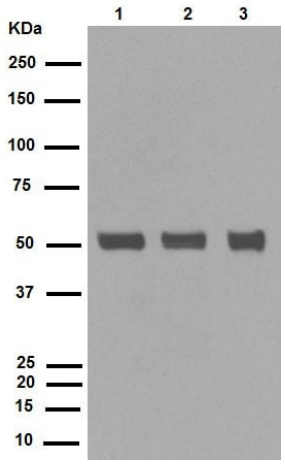
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : Wild-type HAP1 whole cell lysate treated with Staurosporin
Lane 3 : Caspase-8 knockout HAP1 whole cell lysate
Lane 4 : Caspase-8 knockout HAP1 whole cell lysate treated with Staurosporin

Lysates/proteins at 20 µg per lane.

Predicted band size: 55 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab32397 observed at 55, 43/41 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab32397 was shown to specifically react with HAP1 + Staurosproin when HAP1 + Staurosproin knockout samples were used. Wild-type and HAP1 + Staurosproin knockout samples were subjected to SDS-PAGE. Ab32397 and **ab9484** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/500 dilution and 1/20000 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.



Western blot - Anti-Caspase-8 antibody [E7] (ab32397)

All lanes : Anti-Caspase-8 antibody [E7] (ab32397) at 1/1000 dilution

Lane 1 : Jurkat cell lysate
Lane 2 : HeLa cell lysate
Lane 3 : IM-9 cell lysate

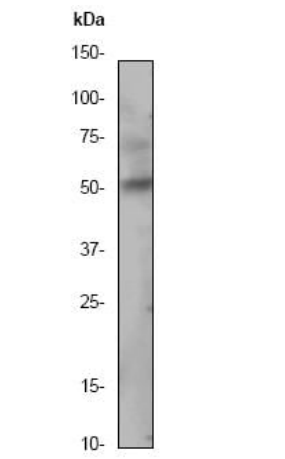
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), HRP-conjugated at 1/1000 dilution

Predicted band size: 55 kDa

Blocking buffer and concentration: 5% NFDM/TBST.

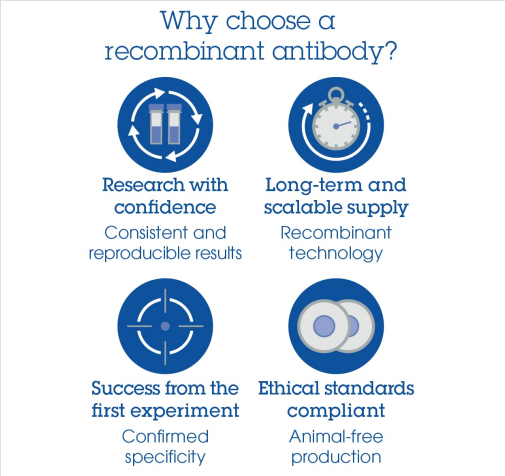
Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-Caspase-8 antibody [E7] (ab32397)

Anti-Caspase-8 antibody [E7] (ab32397) at 1/500 dilution (unpurified) + HeLa cell lysate at 10 µg

Predicted band size: 55 kDa
Observed band size: 55 kDa



Anti-Caspase-8 antibody [E7] (ab32397)

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