abcam

Product datasheet

Human CASP8 (Caspase-8) knockout HeLa cell lysate ab256857

3 Images

Overview

Product name	Human CASP8 (Caspase-8) knockout HeLa cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 2 bp deletion in exon 3.
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. **Usage of SDS sample buffer is not recommended with these lyophilized lysates.*
Notes	**Lysate preparation:** Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are prepared. **User storage instructions:** Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage. Access thousands of knockout cell lysates, generated from commonly used cancer cell lines. **See here for more information on knockout cell lysates.** 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. This product is subject to limited use licenses from The Broad Institute and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant patents please refer to our **limited use license** and **patent pages**.
Tested applications	**Suitable for:** WB

Properties

Storage instructions Store at -80°C. Please refer to protocols.

Components	1 kit
ab261930 - Human CASP8 knockout HeLa cell lysate	1 x 100µg
ab255552 - Human wild-type HeLa cell lysate	1 x 100µg

Cell type epithelial

Disease Adenocarcinoma

Gender Female

STR Analysis Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18 TH01: 7 TPOX: 8, 12 CSF1PO: 9, 10

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.

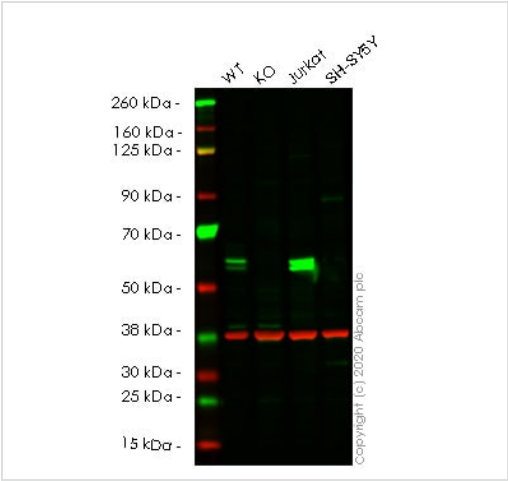
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab256857 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		Use at an assay dependent concentration. Predicted molecular weight: 55 kDa.

Images



Western blot - Human CASP8 (Caspase-8) knockout HeLa cell lysate (ab256857)

Lane 1: Wild-type HeLa cell lysate (20µg)

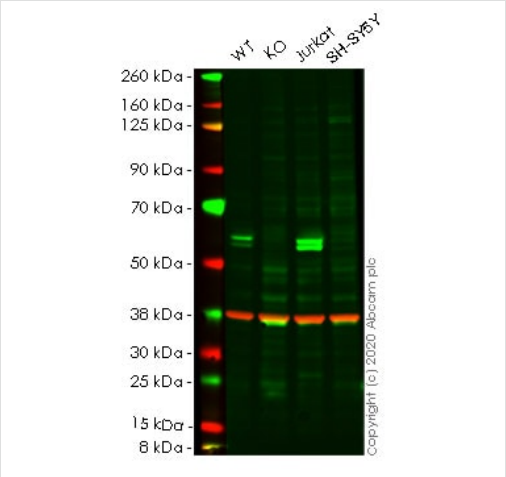
Lane 2: CASP8 knockout HeLa cell lysate (20µg)

Lane 3: Jurkat cell lysate (20µg)

Lane 4: SH-SY5Y cell lysate (20µg)

Lanes 1- 4: Merged signal (red and green). Green - **ab32397** observed at 55 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab32397 Rabbit monoclonal [EPR2418Y] to IRF3 was shown to specifically 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 and Caspase-8 knockout samples 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**) were incubated overnight at 4°C at 1 in 500 and 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 - Human CASP8 (Caspase-8) knockout HeLa cell lysate (ab256857)

Lane 1: Wild-type HeLa cell lysate (20µg)

Lane 2: CASP8 knockout HeLa cell lysate (20µg)

Lane 3: Jurkat cell lysate (20µg)

Lane 4: SH-SY5Y cell lysate (20µg)

Lanes 1- 4: Merged signal (red and green). Green - **ab32125** observed at 55 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab32125 Anti-Caspase-8 antibody [E6] was shown to specifically 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 and Caspase-8 knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab32125** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 3000 and 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.

Sanger Sequencing - Human CASP8 knockout HeLa cell lysate (ab256857)

Homozygous: 2 bp deletion in exon 3

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