abcam

Product datasheet

Human CASP8 (Caspase-8) knockout HeLa cell pellet ab278891

3 Images

Overview

Product name	Human CASP8 (Caspase-8) knockout HeLa cell pellet
Product overview	Abcam's knockout cell pellets give you access to native proteins, without the need to culture cells. Our knockout cell pellets are prepared from our single-gene knockout cell lines and provide an additional offering to our cell lysates. Cells are snap-frozen to provide high quality pellets that are suitable for extraction with alternative lysis buffers or for preparation of lysates from subcellular fractions. Our knockout cell pellets are suitable for a variety of applications, including PCR, gene expression profiling and DNA library preparation.
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)
Notes	**Pellet size:** 5 million cells/vial. 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
Human CASP8 knockout HeLa cell pellet	1 vial
Human wild-type HeLa cell pellet	1 vial

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.

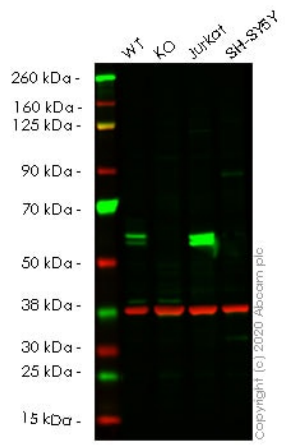
Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab278891 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 pellet (ab278891)

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

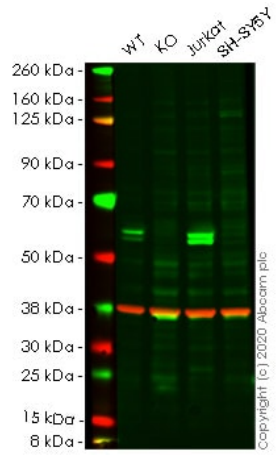
Lane 2: CASP8 knockout HeLa cell lysate (20µg)

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 pellet (ab278891)

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 (Caspase-8) knockout HeLa cell pellet (ab278891)

Homozygous: 2 bp deletion in exon 3

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