abcam

Product datasheet

Anti-pro Caspase-3 antibody [E61] - BSA and Azide free ab183179

敲除验证 重组 RabMAb

7 References 7 图像

概述

产品名称	Anti-pro Caspase-3抗体[E61] - BSA and Azide free
描述	兔单克隆抗体[E61] to pro Caspase-3 - BSA and Azide free
宿主	Rabbit
特异性	The antibody only recognizes the pro-form of Caspase-3. It does not react with the cleaved forms (active enzyme) of Caspase-3.
经测试应用	**适用于:** Flow Cyt (Intra), ICC/IF, IP, IHC-P, WB
种属反应性	**与反应:** Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: Hap1 cells. ICC/IF: Jurkat cells. IHC-P: cervical carcinoma tissue. IP: HeLa whole cell lysate. Flow Cyt (Intra): Jurkat cells.
常规说明	ab183179 is the carrier-free version of **ab32150**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C. Do Not Freeze.
存储溶液	pH: 7.20 Constituent: PBS
无载体	**是**
纯度	Protein A purified
克隆	单克隆
克隆编号	E61
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab183179于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 35 kDa (predicted molecular weight: 31 kDa).

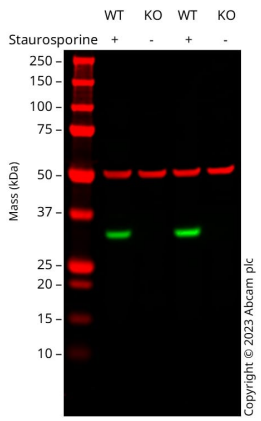
靶标

相关性 Caspases are a family of cysteine proteases that are key mediators of programmed cell death or apoptosis. The precursor form of all caspases is composed of a prodomain, and large and small catalytic subunits. The active forms of caspases are generated by several stimuli including ligand-receptor interactions, growth factor deprivation and inhibitors of cellular functions. All known caspases require cleavage adjacent to aspartates to liberate one large and one small subunit, which associate into a2b2 tetramer to form the active enzyme. Gene for Caspase 3 also known as Yama, CPP32, and apopain codes for a 32-kDa protein. Caspase 3 cleaves the death

substrate poly(ADP-ribose) polymerase (PARP) to a specific 85 kDa form observed during apoptosis and is inhibitable by the CrmA protein. Other Caspase 3 substrates include DNA-PK, actin, GAS2, and procaspase-6, etc. Caspase 3 is activated by cleavage events at Asp-28/Ser-29 (between N-terminal pro-domain) and Asp-175/Ser-176 (between large and small subunits) to generate a large subunit of 17-kDa and a small subunit of 12-kDa.

细胞定位 Cytoplasmic

图片



Western blot - Anti-pro Caspase-3 antibody [E61] - BSA and Azide free (ab183179)

All lanes : Anti-pro Caspase-3 antibody [E61] (**ab32150**) at 1/1000 dilution

Lane 1 : Wild-type HeLa Treated Staurosporine (2uM, 4h) cell lysate
Lane 2 : CASP3 knockout HeLa Treated Staurosporine (2uM, 4h) cell lysate
Lane 3 : Wild-type HeLa Vehicle Control Staurosporine (0uM, 4h) cell lysate
Lane 4 : CASP3 knockout HeLa Vehicle Control Staurosporine (0uM, 4h) cell lysate

Lysates/proteins at 20 µg per lane.

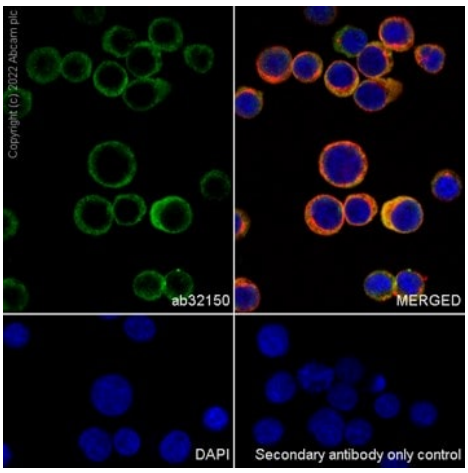
Performed under reducing conditions.

Predicted band size: 31 kDa
Observed band size: 30 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32150**).

Anti-CASP3 antibody [E61] (**ab32150**) staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab32150** was shown to bind specifically to CASP3. A band was observed at 30 kDa in treated wild-type HeLa cell lysates with no signal observed at this size in CASP3 knockout cell line. To generate this image, wild-type and CASP3 knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were

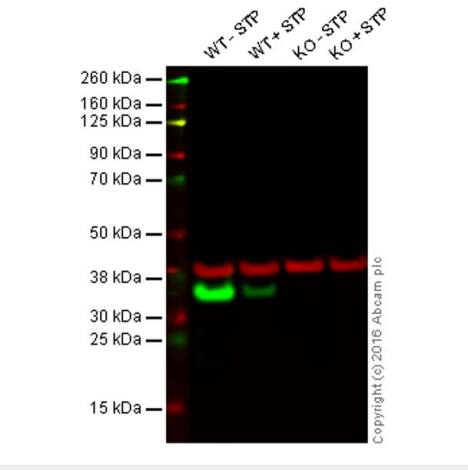
washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-pro Caspase-3 antibody [E61] - BSA and Azide free (ab183179)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Jurkat (Human T cell leukemia cell line from peripheral blood) cells labelling pro Caspase-3 with primary antibody anti-pro Caspase-3 (**ab32150**) at 1/200 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150081**) secondary antibody at 1/1000 dilution. Confocal image showing cytoplasmic staining in Jurkat cells. Anti-alpha Tubulin antibody (DM1A) - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) was used to counterstain tubulin at 1/200 dilution. The nuclear counter stain is DAPI (blue). Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32150**).

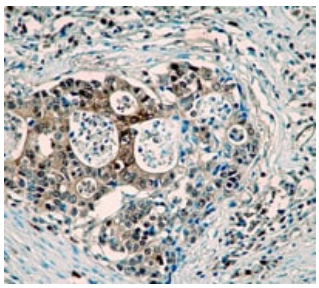


Western blot - Anti-pro Caspase-3 antibody [E61] - BSA and Azide free (ab183179)

This WB data was generated using the same anti-pro Caspase-3 antibody clone, E61, in a different buffer formulation (**ab32150**).

Lane 1: Wild-type HAP1 cell lysate
Lane 2: Wild-type HAP1 cell lysate + Staurosporine (1µM for 4h)
Lane 3: Caspase-3 knockout HAP1 cell lysate
Lane 4: Caspase-3 knockout HAP1 cell lysate + Staurosporine (1µM for 4h)
Lanes 1 - 4: Merged signal (red and green). Green - **ab32150** observed at 35 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab32150 was shown to specifically react with pro Caspase 3 when Caspase 3 knockout samples were used. Wild-type and Caspase 3 knockout samples (± Staurosporine treatment) were subjected to SDS-PAGE. **ab32150** and **ab8245** (loading control to GAPDH) were diluted to 1/1000 and 1/10000 respectively and incubated overnight at 4°C. 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 1/10000 dilution for 1 hour at room temperature before imaging.

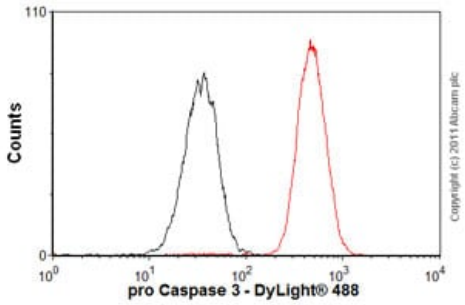


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-pro Caspase-3 antibody [E61] - BSA and Azide free (ab183179)

Immunohistochemical analysis of human paraffin-embedded cervical carcinoma tissue using **ab32150** at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32150**).

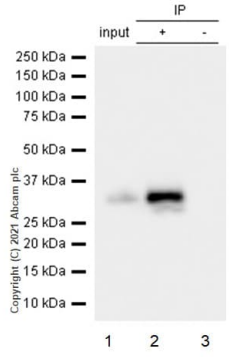
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-pro Caspase-3 antibody [E61] - BSA and Azide free (ab183179)

Overlay histogram showing Jurkat cells stained with **ab32150** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions. The cells were then incubated with the antibody (**ab32150**, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was anti-rabbit DyLight® 488 (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in Jurkat cells fixed with 4% paraformaldehyde (10 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32150**).



Immunoprecipitation - Anti-pro Caspase-3 antibody [E61] - BSA and Azide free (ab183179)

This data was developed using **ab32150**, the same antibody clone in a different buffer formulation.

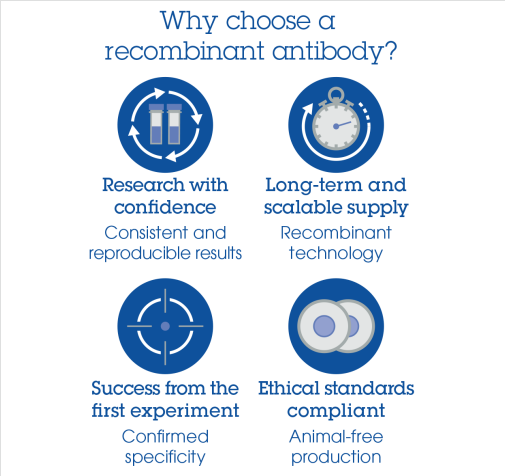
pro Caspase-3 was immunoprecipitated from 0.35 mg HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg with **ab32150** at 1/20 dilution (0.6µg). VeriBlot for IP Detection Reagent (HRP)(**ab131366**) was used at 1/5000 dilution.

Lane 1: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg

Lane 2: abab32150 IP in HeLa whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab32150** in HeLa whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.



Anti-pro Caspase-3 antibody [E61] - BSA and Azide free (ab183179)

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