

Anti-pro Caspase-3 antibody [E61] ab32150

KO 評価済 リコンビナント RabMAb

★★★★★ [2 Abreviews](#) [132 References](#) [画像数 8](#)

製品の概要

製品名	Anti-pro Caspase-3 antibody [E61]
製品の詳細	Rabbit monoclonal [E61] to pro Caspase-3
由来種	Rabbit
特異性	The antibody only recognizes the pro-form of Caspase-3. It does not react with the cleaved forms (active enzyme) of Caspase-3.
アプリケーション	適用あり: IP, WB, IHC-P, ICC/IF, Flow Cyt (Intra)
種交差性	交差種: Human
免疫原	Synthetic peptide within Human pro Caspase-3 (N terminal). The exact sequence is proprietary.
ポジティブ・コントロール	WB: Hap1 & HeLa cells. ICC/IF: Jurkat cells. IHC-P: cervical carcinoma tissue. IP: HeLa whole cell lysate. Flow Cyt (Intra): Jurkat cells.
特記事項	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p> <p>Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.</p>

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
バッファー	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
精製度	Protein A purified

ポリ/モノ	モノクローナル
クローン名	E61
アイソタイプ	IgG

アプリケーション

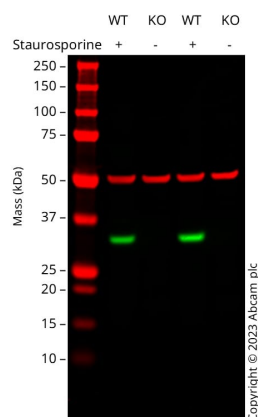
The Abpromise guarantee **Abpromise保証は、次のテスト済みアプリケーションにおけるab32150の使用に適用されます**
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご確認ください。

アプリケーション	Abreviews	特記事項
IP		1/20.
WB	★★★★★ (2)	1/1000. Detects a band of approximately 35 kDa.
IHC-P		1/500. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		1/200.
Flow Cyt (Intra)		1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

ターゲット情報

関連性	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.
細胞内局在	Cytoplasmic

画像



Western blot - Anti-pro Caspase-3 antibody [E61]
(ab32150)

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

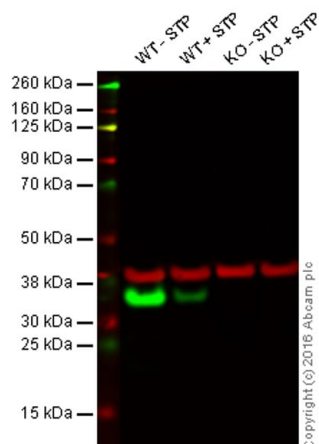
Lane 4 : CASP3 knockout HeLa Vehicle Control Staurosporine (0uM, 4h) cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 30 kDa

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.



Western blot - Anti-pro Caspase-3 antibody [E61]
(ab32150)

Lane 1: Wild-type HAP1 cell lysate

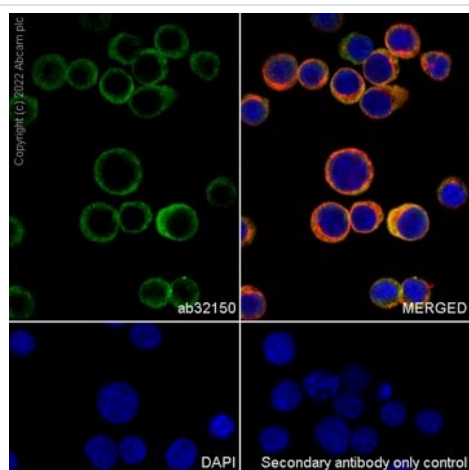
Lane 2: Wild-type HAP1 cell lysate + Staurosporine (1μM for 4h)

Lane 3: Caspase-3 knockout HAP1 cell lysate

Lane 4: Caspase-3 knockout HAP1 cell lysate + Staurosporine (1μM for 4h)

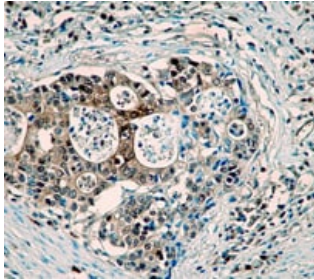
Lanes 1 - 4: Merged signal (red and green). Green - ab32150 observed at 35 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

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.



Immunocytochemistry/ Immunofluorescence - Anti-pro Caspase-3 antibody [E61] (ab32150)

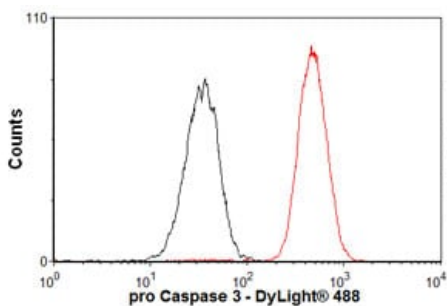
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Jurkat (Human T cell leukemia cell line from peripheral blood) cells labelling pro Caspase-3 with primary antibody anti-pro Caspase-3 (ab32150) at 1/200 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 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).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-pro Caspase-3 antibody [E61] (ab32150)

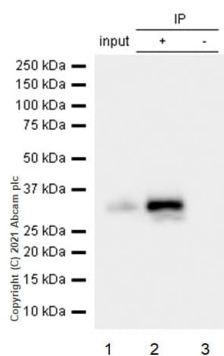
Immunohistochemical analysis of human paraffin-embedded cervical carcinoma tissue using ab32150 at 1/500 dilution.

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] (ab32150)

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/1x10⁶ 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.



Immunoprecipitation - Anti-pro Caspase-3 antibody
[E61] (ab32150)

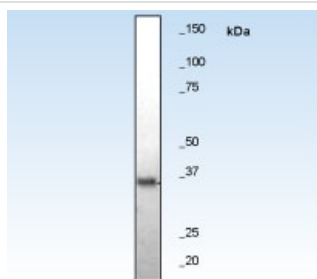
pro Caspase-3 was immunoprecipitated from 0.35 mg HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg with ab32150 at 1/20 dilution (0.6µg). VeriBlot for IP Detection Reagent (HRP)([ab131366](#)) was used at 1/5000 dilution.

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

Lane 2: ab32150 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.



Western blot - Anti-pro Caspase-3 antibody [E61]
(ab32150)

Anti-pro Caspase-3 antibody [E61] (ab32150) at 1/1000 dilution + HeLa cell lysate

Observed band size: 35 kDa

Why choose a recombinant antibody?



Research with confidence
Consistent and reproducible results



Long-term and scalable supply
Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

Anti-pro Caspase-3 antibody [E61] (ab32150)

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