abcam

Product datasheet

Anti-pro Caspase-3 antibody [E83-103] ab32499

敲除验证 重组 RabMAb

79 References 6 图像

概述

产品名称	Anti-pro Caspase-3抗体[E83-103]
描述	兔单克隆抗体[E83-103] to pro Caspase-3
宿主	Rabbit
特异性	This antibody only detects pro-form (35kD) of caspase-3, and does not recognize any cleaved caspases.
经测试应用	**适用于:** ICC/IF, WB, IHC-P, Flow Cyt (Intra)
种属反应性	**与反应:** Mouse, Human
免疫原	Synthetic peptide corresponding to Human pro Caspase-3 (N terminal).
阳性对照	Jurkat whole cell lysate (**ab7899**) and human colon adenocarcinoma.
常规说明	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**. Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

性能

形式	Liquid
存放说明	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified
克隆	单克隆

克隆编号	E83-103
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab32499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。

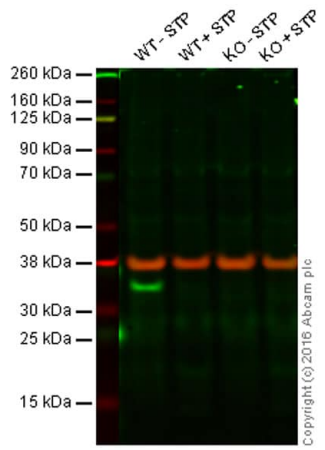
应用	Ab评论	说明
ICC/IF		Use a concentration of 5 µg/ml.
WB		1/10000. Detects a band of approximately 35 kDa (predicted molecular weight: 31 kDa).
IHC-P		1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/50. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

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

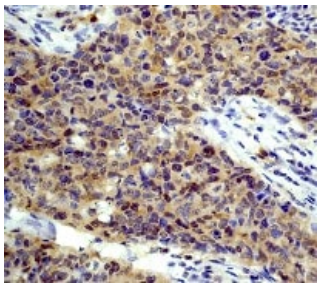
图片



Western blot - Anti-pro Caspase-3 antibody [E83-103] (ab32499)

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 - ab32499 observed at 35 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

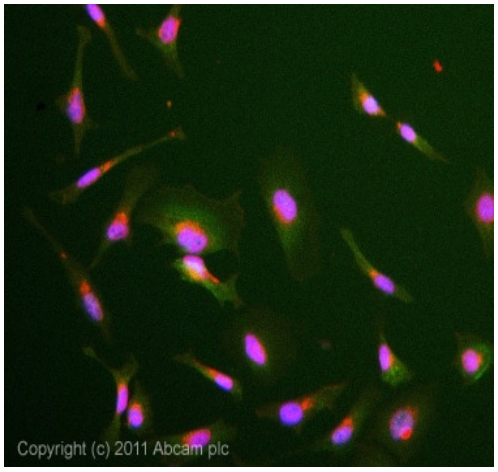
ab32499 was shown to specifically react with pro Caspase 3 when Caspase 3 knockout samples were used. Wild-type and Caspase 3 knockout samples (± Staurosporine treatment) were subjected to SDS-PAGE. ab32499 and **ab8245** (loading control to GAPDH) were diluted to 1/1000 and 1/10000 respectively and 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 [E83-103] (ab32499)

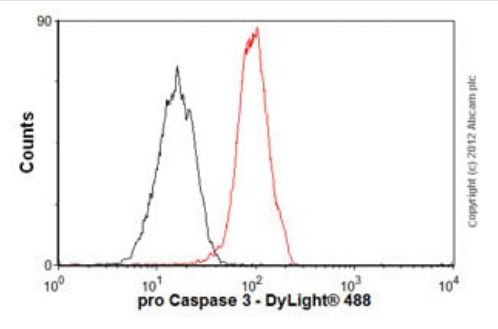
Immunohistochemical analysis of paraffin-embedded human colon adenocarcinoma ab32499 at 1/250 dilution.

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



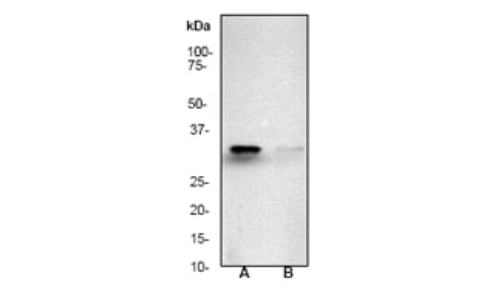
Immunocytochemistry/ Immunofluorescence - Anti-pro Caspase-3 antibody [E83-103] (ab32499)

ICC/IF image of ab32499 stained HeLa cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab32499, 5µg/ml) overnight at +4°C. The secondary antibody (green) was **ab96899**, anti-rabbit DyLight® 488 used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Flow Cytometry (Intracellular) - Anti-pro Caspase-3 antibody [E83-103] (ab32499)

Overlay histogram showing Jurkat cells stained with ab32499 (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 followed by the antibody (ab32499, 1/50 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 (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.



Western blot - Anti-pro Caspase-3 antibody [E83-103] (ab32499)

All lanes : Anti-pro Caspase-3 antibody [E83-103] (ab32499) at 1/10000 dilution

Lane 1 : Jurkat cell lysate
Lane 2 : Jurkat cell lysate + Camptothecin

Predicted band size: 31 kDa
Observed band size: 35 kDa

Anti-pro Caspase-3 antibody [E83-103] (ab32499)

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