abcam

Product datasheet

Anti-IKK beta antibody [Y466] ab32135

敲除验证 重组 RabMAb

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概述

产品名称	Anti-IKK beta抗体[Y466]
描述	兔单克隆抗体[Y466] to IKK beta
宿主	Rabbit
经测试应用	**适用于:** WB, IP
种属反应性	**与反应:** Mouse, Human
免疫原	Synthetic peptide within Human IKK beta aa 650-750 (C terminal). The exact sequence is proprietary. Database link: **O14920**
阳性对照	WB: Wild-type HAP1 cell lysate. HeLa, NIH/3T3 and Daudi cell lysate. IHC-P: Human cervical carcinoma tissue. IP: SH-SY5Y whole cell lysate.
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. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
纯度	Protein A purified

克隆	单克隆
克隆编号	Y466
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab32135于以下的经测试应用

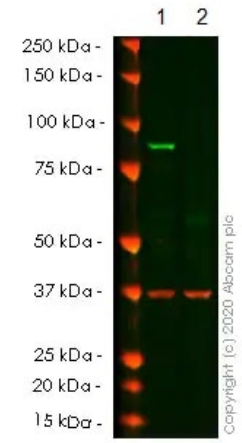
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB	★★★★★ (3)	1/1000 - 1/5000. Detects a band of approximately 87 kDa. This antibody has weaker binding with mouse than human.
IP		1/50.

靶标

功能	Acts as part of the IKK complex in the conventional pathway of NF-kappa-B activation and phosphorylates inhibitors of NF-kappa-B thus leading to the dissociation of the inhibitor/NF-kappa-B complex and ultimately the degradation of the inhibitor. Also phosphorylates NCOA3.
组织特异性	Highly expressed in heart, placenta, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis and peripheral blood.
序列相似性	Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. I-kappa-B kinase subfamily. Contains 1 protein kinase domain.
翻译后修饰	Upon cytokine stimulation, phosphorylated on Ser-177 and Ser-181 by MEKK1 and/or MAP3K14/NIK; which enhances activity. Once activated, autophosphorylates on the C-terminal serine cluster; which decreases activity and prevents prolonged activation of the inflammatory response. Acetylation of Thr-180 by Yersinia yopJ prevents phosphorylation and activation, thus blocking the I-kappa-B pathway. Ubiquitinated. Monoubiquitination involves TRIM21 that leads to inhibition of Tax-induced NF-kappa-B signaling. According to PubMed:19675099, 'Ser-163' does not serve as a monoubiquitination site. According to PubMed:16267042, ubiquitination on 'Ser-163' modulates phosphorylation on C-terminal serine residues. Monoubiquitination by TRIM21 is dirupted by Yersinia yopJ.
细胞定位	Cytoplasm. Membrane raft. Colocalized with DPP4 in membrane rafts.

图片



Western blot - Anti-IKK beta antibody [Y466] (ab32135)

All lanes : Anti-IKK beta antibody [Y466] (ab32135) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : IKBKB CRISPR/Cas9 edited HeLa cell lysate

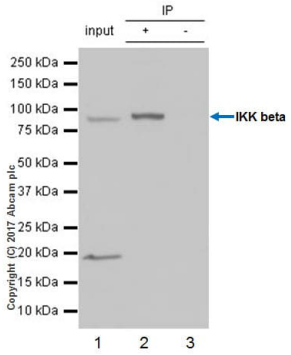
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 87 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab32135 observed at 87 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab32135 was shown to react with IKK beta in wild-type HeLa cells in western blot. The band observed in CRISPR/Cas9 edited cell line **ab264847** (CRISPR/Cas9 edited cell lysate **ab257228**) lane below 87kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HeLa and IKBKB CRISPR/Cas9 edited HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab32135 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at a 1 in 1000 dilution and a 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.

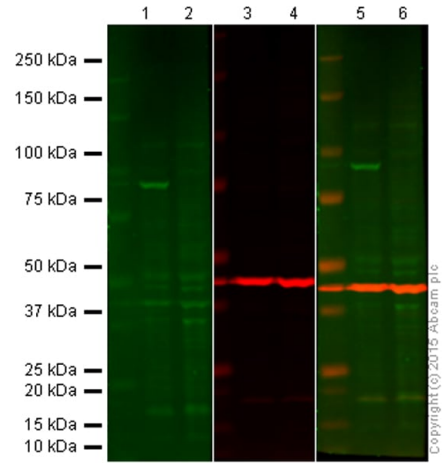


Immunoprecipitation - Anti-IKK beta antibody [Y466] (ab32135)

ab32135 (purified) at 1:50 dilution (2µg) immunoprecipitating IKK beta in SH-SY5Y (Human neuroblastoma epithelial cell) whole cell lysate.

Lane 1 (input): SH-SY5Y (Human neuroblastoma epithelial cell) whole cell lysate 10ug
Lane 2 (+): ab32135 & SH-SY5Y (Human neuroblastoma epithelial cell) whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32135 in SH-SY5Y (Human neuroblastoma epithelial cell) whole cell lysate

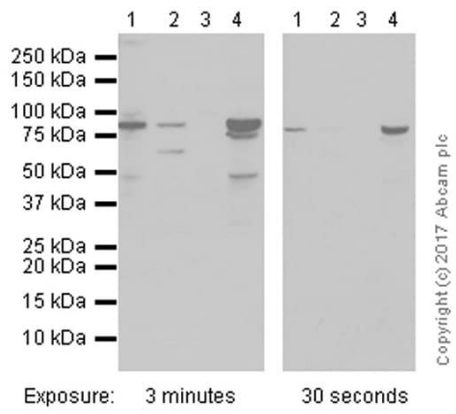
For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1:10,000 dilution.
Blocking and diluting buffer: 5% NFDM/TBST.



Western blot - Anti-IKK beta antibody [Y466] (ab32135)

Lanes 1, 3 and 5: Wild-type HAP1 cell lysate (20 µg)
Lanes 2, 4 and 6: IKK beta knockout HAP1 cell lysate (20 µg)
Lanes 1 and 2: Green signal from target – ab32135 observed at 87 kDa
Lanes 3 and 4: Red signal from loading control – **ab8226** observed at 42 kDa
Lanes 5 and 6: Merged (red and green) signal

Unpurified ab32135 was shown to react with IKK beta when IKK beta knockout samples were used, along with additional cross-reactive bands. Wild-type and IKK beta knockout samples were subjected to SDS-PAGE. ab32135 and **ab8226** (loading control to beta actin) were diluted 1/1000 and 1/2000 respectively and incubated overnight at 4ºC. 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 1/10 000 dilution for 1 h at room temperature before imaging.



Western blot - Anti-IKK beta antibody [Y466] (ab32135)

All lanes : Anti-IKK beta antibody [Y466] (ab32135) at 1/1000 dilution (purified)

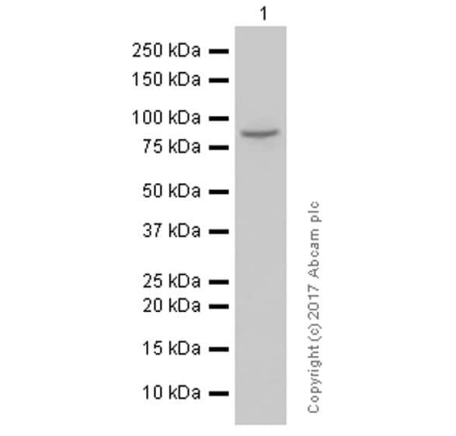
Lane 1 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates
Lane 2 : Mouse lung lysates
Lane 3 : Rat brain lysates
Lane 4 : SH-SY5Y (Human neuroblastoma epithelial cell) whole cell lysates

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Observed band size: 87 kDa

Blocking and diluting buffer: 5% NFDM/TBST

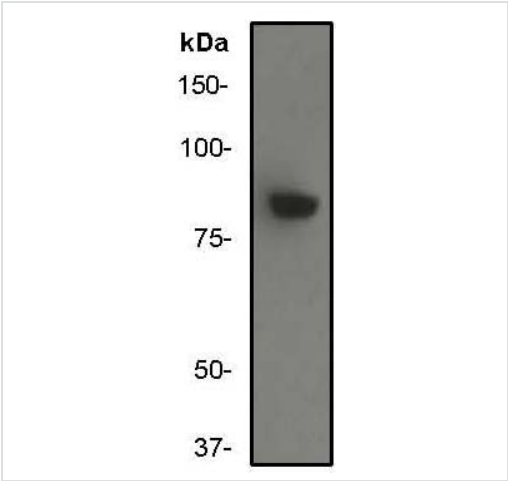


Western blot - Anti-IKK beta antibody [Y466] (ab32135)

Anti-IKK beta antibody [Y466] (ab32135) at 1/2500 dilution (purified) + HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates at 20 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

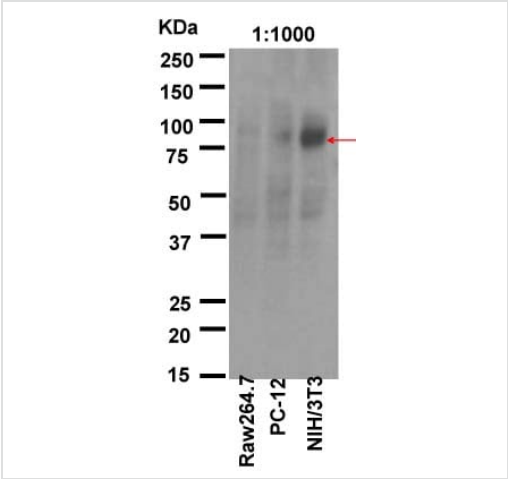
Blocking and diluting buffer: 5% NFDM/TBST



Western blot - Anti-IKK beta antibody [Y466] (ab32135)

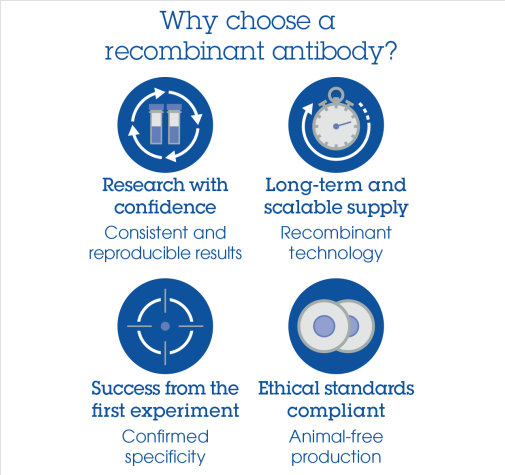
Anti-IKK beta antibody [Y466] (ab32135) at 1/5000 dilution (unpurified) + Daudi cell lysate

Observed band size: 87 kDa



Western blot - Anti-IKK beta antibody [Y466] (ab32135)

unpurified ab32135



Anti-IKK beta antibody [Y466] (ab32135)

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