abcam

Product datasheet

Anti-IKK beta antibody [Y466] ab32135



ייבעדיו RabMAb

★★★★★ 3 Abreviews 44 References 画像数8

製品の概要

製品名 Anti-IKK beta antibody [Y466]

製品の詳細 Rabbit monoclonal [Y466] to IKK beta

由来種 Rabbit

アプリケーション **適用あり:** WB, IP

種交差性 交差種: Mouse, Human

免疫原 Synthetic peptide within Human IKK beta aa 650-750 (C terminal). The exact sequence is

proprietary.

Database link: **O14920**

ポジティブ・コントロール 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.

特記事項 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.

製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

pH: 7.20 バッファー

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

精製度 Protein A purified

ポリ/モノ モノクローナル

ウローン名 Y466 アイソタイプ IgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab32135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検討ください。

アプリケーション	Abreviews	特記事項
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.

ターゲット情報

機能 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.

組織特異性 Highly expressed in heart, placenta, skeletal muscle, kidney, pancreas, spleen, thymus, prostate,

testis and peripheral blood.

配列類似性 Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. I-kappa-B kinase

subfamily.

Contains 1 protein kinase domain.

翻訳後修飾 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-

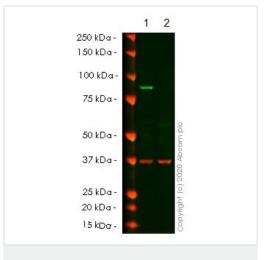
kappa-B signaling. According to PubMed:19675099, 'Ser-163' does not serve as a

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.

細胞内局在 Cytoplasm. Membrane raft. Colocalized with DPP4 in membrane rafts.

画像



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

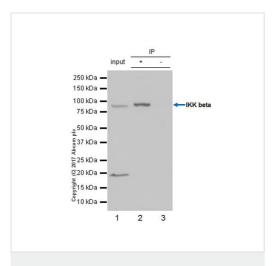
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

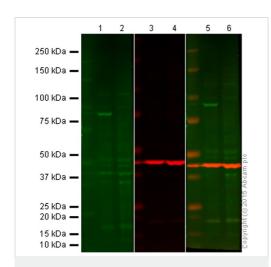
Observed band size: 87 kDa

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)



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

ab32135 (purified) at 1:50 dilution (2 μ g) 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 (<u>ab172730</u>) 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.

Lanes 1, 3 and 5: Wild-type HAP1 cell lysate (20 μ g)

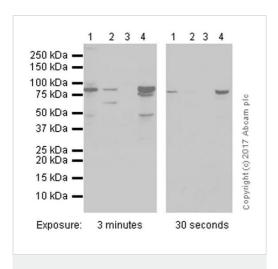
Lanes 2, 4 and 6: IKK beta knockout HAP1 cell lysate (20 µg)

Lanes 1 and 2: Green signal from target – ab32135 observed at 87 kDa

Lanes 3 and 4: Red signal from loading control – <u>ab8226</u> 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 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 1/10 000 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

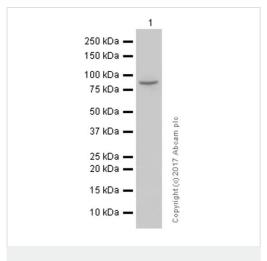
Lysates/proteins at 20 µg per lane.

Secondary

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

Observed band size: 87 kDa

Blocking and diluting buffer: 5% NFDM/TBST



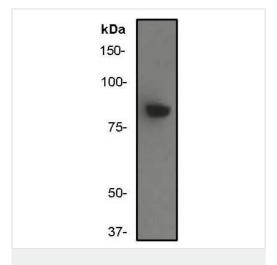
Western blot - Anti-IKK beta antibody [Y466] (ab32135)

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

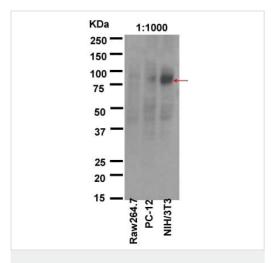
Blocking and diluting buffer: 5% NFDM/TBST



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

Observed band size: 87 kDa





unpurified ab32135

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



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