abcam

Product datasheet

Anti-IKK beta antibody [Y466] - BSA and Azide free ab171363



יטלאעבע RabMAb

画像数 4

製品の概要

特記事項

製品名 Anti-IKK beta antibody [Y466] - BSA and Azide free

製品の詳細 Rabbit monoclonal [Y466] to IKK beta - BSA and Azide free

由来種 Rabbit

アプリケーション **適用あり:** IP, WB

種交差性 交差種: Mouse. Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール WB: Wild-type HAP1 and HeLa cell lysate.

ab171363 is the carrier-free version of ab32135.

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 cellbased 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.

製品の特性

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製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C. Do Not Freeze.

パッファー Constituent: PBS

キャリア・フリー はい

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 IqG

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ab171363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
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 87 kDa.

ターゲット情報

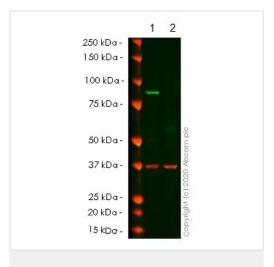
機能	Acts as part of the IKK complex in the conventional pathway of NF-kappa-B activation and phosphorylates inhibitors of NF-kappa-B thus leading to the dissociation of the inhibitor/NF-kappa-B complex and ultimately the degradation of the inhibitor. Also phosphorylates NCOA3.
組織特異性	Highly expressed in 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

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

Yersinia yopJ.



Western blot - Anti-IKK beta antibody [Y466] - BSA and Azide free (ab171363)

All lanes : Anti-IKK beta antibody [Y466] (<u>ab32135</u>) 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.

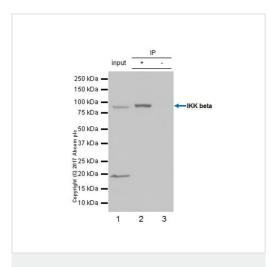
Performed under reducing conditions.

Predicted band size: 87 kDa **Observed band size:** 87 kDa

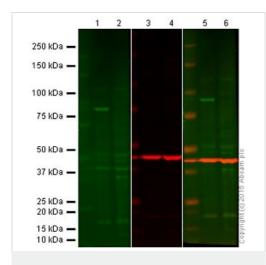
This data was developed using the same antibody clone in a different buffer formulation (<u>ab32135</u>).

Lanes 1-2: Merged signal (red and green). Green - <u>ab32135</u> observed at 87 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) 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] - BSA and Azide free (ab171363)



Western blot - Anti-IKK beta antibody [Y466] - BSA and Azide free (ab171363)

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 (+): <u>ab32135</u> & SH-SY5Y (Human neuroblastoma epithelial cell) whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of <u>ab32135</u> 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32135).

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 μ g)

Lanes 1 and 2: Green signal from target – <u>ab32135</u> 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 <u>ab32135</u> 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. <u>ab32135</u> and <u>ab8226</u> (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 (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32135).



(ab171363)

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