abcam

Product datasheet

Anti-IKK alpha antibody [Y463] ab32041



★★★★ 6 Abreviews 67 References

画像数9

製品の概要

製品名 Anti-IKK alpha antibody [Y463]

製品の詳細 Rabbit monoclonal [Y463] to IKK alpha

由来種 Rabbit

特異性 The rat recommendation is based on the WB results. We do not guarantee IHC-P for rat.

アプリケーション 適用あり: Flow Cyt (Intra), WB, IHC-P, IP

種交差性 交差種: Mouse. Rat. 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: HAP1-WT; Daudi, RAW 264.7 and C6 whole cell lysate. Mouse and rat kidney lysate. Flow

Cyt (intra): HAP1-WT and Daudi cells; IP: HeLa cell lysate; IHC-P: Human ovarian cancer and

mouse kidney tissue.

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**.

製品の特件

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 long

term. Avoid freeze / thaw cycle.

バッファー pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

精製度 Protein A purified

ポリモノ モノクローナル

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ab32041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
Flow Cyt (Intra)		1/20. For unpurified use at 1/50. <u>ab172730</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB	★★★★ (4)	1/1000. Detects a band of approximately 88 kDa (predicted molecular weight: 85 kDa). For unpurified use at 1/10000 - 1/50000.
IHC-P		1/50. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. See IHC antigen retrieval protocols. The rat recommendation is based on the WB results. We do not guarantee IHC-P for rat.
IP	★★★ ☆☆ (1)	1/60.

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. As part of the non-canonical pathway of NF-kappa-B activation, the MAP3K14-activated CHUK/IKKA homodimer phosphorylates NFKB2/p100 associated with RelB, inducing its proteolytic processing to NFKB2/p52 and the formation of NF-kappa-B RelB-p52 complexes. Also phosphorylates NCOA3. Phosphorylates 'Ser-10' of histone H3 at NF-kappa-B-regulated promoters during

inflammatory responses triggered by cytokines.

組織特異性 Widely expressed.

関連疾患 Defects in CHUK are the cause of cocoon syndrome (COCOS) [MIM:613630]; also known as

fetal encasement syndrome. COCOS is a lethal syndrome characterized by multiple fetal malformations including defective face and seemingly absent limbs, which are bound to the trunk

and encased under the skin.

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.

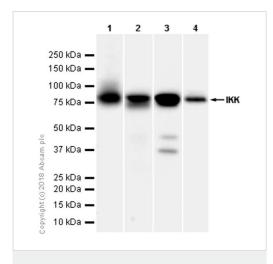
翻訳後修飾 Phosphorylated by MAP3K14/NIK, AKT and to a lesser extent by MEKK1, and dephosphorylated

by PP2A. Autophosphorylated.

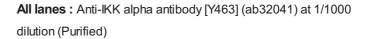
Acetylation of Thr-179 by Yersinia yopJ prevents phosphorylation and activation, thus blocking the

I-kappa-B signaling pathway.

画像



Western blot - Anti-IKK alpha antibody [Y463] (ab32041)



Lane 1 : RAW 264.7 (Mouse Abelson murine leukemia virusinduced tumor macrophage) whole cell lysate

Lane 2: Mouse kidney lysate

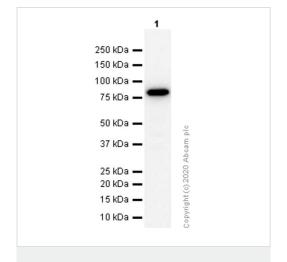
Lane 3: C6 (Rat glial tumor glial cell) whole cell lysate

Lane 4: Rat kidney lysate

Secondary

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

Predicted band size: 85 kDa



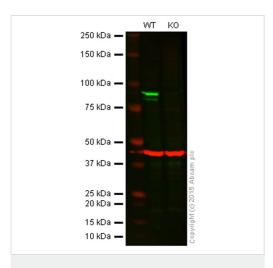
Western blot - Anti-IKK alpha antibody [Y463] (ab32041)

Anti-IKK alpha antibody [Y463] (ab32041) at 1/10000 dilution + Daudi (Human Burkitt's lymphoma lymphoblast) whole cell lysate

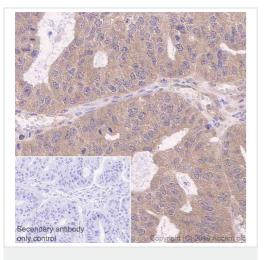
Secondary

Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/20000 dilution

Predicted band size: 85 kDa



Western blot - Anti-IKK alpha antibody [Y463] (ab32041)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IKK alpha antibody [Y463] (ab32041)

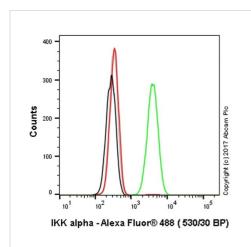
Lane 1: Wild-type HAP1 cell lysate (20 µg)

Lane 2: IKK alpha knockout HAP1 cell lysate (20 µg)

Lanes 1 and 2: Merged signal (red and green). Green - ab32041 observed at 84 kDa. Red - loading control, **ab8226**, observed at 42 kDa.

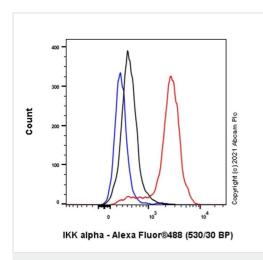
ab32041 (Unpurified format) was shown to specifically react with IKK alpha when IKK alpha knockout samples were used. Wild-type and IKK alpha knockout samples were subjected to SDS-PAGE. ab32041 and <u>ab8226</u> (loading control to beta actin) were diluted 1/10000 and 1/1000 respectively and incubated 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 1/10000 dilution for 1 hour at room temperature before imaging.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human ovarian cancer tissue sections labeling IKK alpha with purified ab32041 at 1/50 dilution (3.92 µg/mL). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) secondary antibody was used at 1/0 dilution. PBS instead of the primary antibody was used as the negative control.



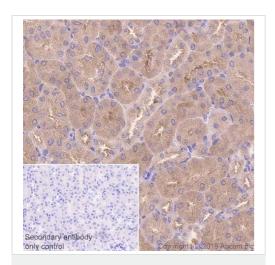
Flow Cytometry (Intracellular) - Anti-IKK alpha antibody [Y463] (ab32041)

Overlay histogram showing HAP1 wildtype (green line) and HAP1-CHUK knockout cells (red line) stained with ab32041. The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab32041, 1µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor[®] 488 goat anti-rabbit lgG (H&L) presorbed (ab150081) at 1/2000 dilution for 30 min at 22°C. A rabbit lgG isotype control antibody (ab172730) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-CHUK knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter. This antibody can also be used in HAP1 cells fixed with 80% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



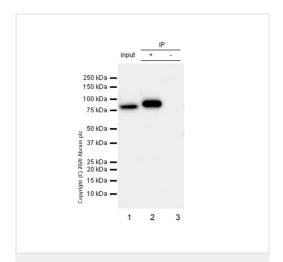
Flow Cytometry (Intracellular) - Anti-IKK alpha antibody [Y463] (ab32041)

Intracellular Flow Cytometry analysis of Daudi (Human Burkitt's lymphoma lymphoblast) cells labelling IKK alpha with purified ab32041 at 1/20 dilution (10 µg/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor[®] 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IKK alpha antibody [Y463] (ab32041)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse kidney tissue sections labeling IKK alpha with purified ab32041 at 1/50 dilution (3.92 µg/mL). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) secondary antibody was used at 1/0 dilution. PBS instead of the primary antibody was used as the negative control.



Immunoprecipitation - Anti-IKK alpha antibody [Y463] (ab32041)

Purified ab32041 at 1/60 dilution ($2\mu g$) immunoprecipitating IKK alpha in HeLa whole cell lysate.

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

Lane 2 (+): ab32041 + HeLa whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of <u>ab169743</u> in HeLa whole cell lysate.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) (1/1000 dilution) was used for Western blotting.

Blocking Buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM/TBST.

Observed band size: 88 kDa



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