abcam

Product datasheet

HRP Anti-IKB alpha antibody [E130] ab202646





1 References 4 Images

Overview

Product name HRP Anti-IKB alpha antibody [E130]

Description HRP Rabbit monoclonal [E130] to IKB alpha

Host species Rabbit HRP Conjugation

Tested applications Suitable for: IHC-P, WB

Species reactivity Reacts with: Mouse, Rat, Human

Predicted to work with: Hamster, Cow, Pig

Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. **Immunogen**

Positive control WB: PC12, NIH3T3, RAW 264.7 whole cell lysates and human fetal liver tissue lysate. IHC:

Human kidney (FFPE)

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit General notes

monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

Properties

Liquid **Form**

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

Stable for 12 months at -20°C. Store In the Dark.

pH: 7.40 Storage buffer

Preservative: 0.1% Proclin 300 Solution

Constituents: PBS, 30% Glycerol (glycerin, glycerine), 1% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number E130

Isotype lgG

Applications

The Abpromise guarantee

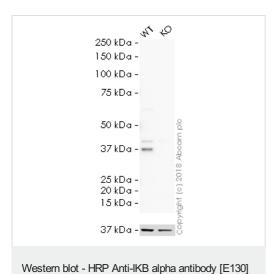
Images

Our <u>Abpromise guarantee</u> covers the use of ab202646 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P		1/50. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		1/5000. Detects a band of approximately 35 kDa (predicted molecular weight: 36 kDa).

Target		
Function	Inhibits the activity of dimeric NF-kappa-B/REL complexes by trapping REL dimers in the cytoplasm through masking of their nuclear localization signals. On cellular stimulation by immune and proinflammatory responses, becomes phosphorylated promoting ubiquitination and degradation, enabling the dimeric RELA to translocate to the nucleus and activate transcription.	
Involvement in disease	Ectodermal dysplasia, anhidrotic, with T-cell immunodeficiency autosomal dominant	
Sequence similarities	Belongs to the NF-kappa-B inhibitor family. Contains 5 ANK repeats.	
Post-translational modifications	Phosphorylated; disables inhibition of NF-kappa-B DNA-binding activity. Phosphorylation at positions 32 and 36 is prerequisite to recognition by UBE2D3 leading to polyubiquitination and subsequent degradation. Sumoylated; sumoylation requires the presence of the nuclear import signal. Sumoylation blocks ubiquitination and proteasome-mediated degradation of the protein thereby increasing the prote stability. Monoubiquitinated at Lys-21 and/or Lys-22 by UBE2D3. Ubiquitin chain elongation is then performed by CDC34 in cooperation with the SCF(FBXW11) E3 ligase complex, building ubiquitin chains from the UBE2D3-primed NFKBIA-linked ubiquitin. The resulting polyubiquitination leads to protein degradation. Also ubiquitinated by SCF(BTRC) following stimulus-dependent phosphorylation at Ser-32 and Ser-36. Deubiquitinated by porcine reproductive and respiratory syndrome virus Nsp2 protein, which thereby interferes with NFKBIA degradation and impairs subsequent NF-kappa-B activation.	
Cellular localization	Cytoplasm. Nucleus. Shuttles between the nucleus and the cytoplasm by a nuclear localization signal (NLS) and a CRM1-dependent nuclear export.	



(ab202646)

All lanes : HRP Anti-IKB alpha antibody [E130] (ab202646) at 1/5000 dilution

Lane 1: Wild-type HAP1 whole cell lysate

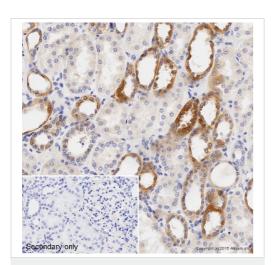
Lane 2: NFKBIA (IKB alpha) knockout HAP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

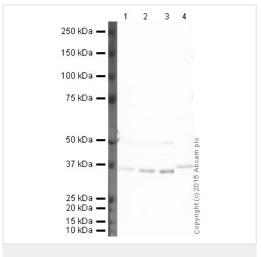
Predicted band size: 36 kDa **Observed band size:** 35 kDa

Exposure time: 12 minutes

ab202646 was shown to recognize IKB alpha in wild-type HAP1 cells as signal was lost at the expected MW in NFKBIA (IKB alpha) knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and NFKBIA (IKB alpha) knockout samples were subjected to SDS-PAGE. Ab202646 and ab184095 (Mouse monoclonal [mAbcam 9484] to GAPDH - Loading Control (Alexa Fluor® 680) loading control) were incubated overnight at 4°C at 1/5000 dilution and 1/1000 dilution respectively. The loading control was imaged using the Licor Odyssey CLx prior to blots being developed with ECL technique.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - HRP Anti-IKB alpha antibody [E130] (ab202646)



Western blot - HRP Anti-IKB alpha antibody [E130] (ab202646)

IHC image of IKB alpha staining in a section of formalin-fixed paraffin-embedded normal human kidney*, performed on a Leica BOND. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab202646, 1/50 dilution, for 15 mins at room temperature. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset negative control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

All lanes : HRP Anti-IKB alpha antibody [E130] (ab202646) at 1/5000 dilution

Lane 1 : PC12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate

Lane 2: NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate

Lane 3 : RAW 264.7 (Mouse leukaemic monocyte macrophage cell line) Whole Cell Lysate

Lane 4: Liver (Human) Tissue Lysate - fetal normal tissue

Lysates/proteins at 10 µg per lane.

Developed using the ECL technique.

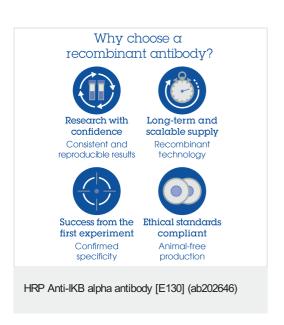
Performed under reducing conditions.

Predicted band size: 36 kDa

Observed band size: 36 kDa

Exposure time: 4 minutes

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% milk before being incubated with ab202646 overnight at 4°C. Antibody binding was visualised using ECL development solution **ab133406**.



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