abcam

Product datasheet

Anti-Rel B antibody [EP614Y] ab33907

KO 評価済 リコンビナント RabMAb

3 References 画像数 9

製品の概要

製品名	Anti-Rel B antibody [EP614Y]
製品の詳細	Rabbit monoclonal [EP614Y] to Rel B
由来種	Rabbit
アプリケーション	**適用あり:** Flow Cyt (Intra), ICC/IF, WB, IP **適用なし:** ChIP or IHC-P
種交差性	**交差種:** Human
免疫原	Synthetic peptide within Human Rel B aa 550-650 (C terminal). The exact sequence is proprietary.
ポジティブ・コントロール	WB: HeLa, Raji and Daudi cell lysate. IP: Daudi cell lysate, Raji whole cell lysate ICC/IF: Raji cells. Flow Cyt (intra): Raji cells.
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**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these 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.
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
クローン名	EP614Y
アイソタイプ	IgG

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ab33907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)		1/70. For unpurified, use 1/1000. **ab172730** - Rabbit monoclonal IgG is suitable for use as an isotype control with this antibody.
ICC/IF		1/100.
WB		1/2000 - 1/20000. Predicted molecular weight: 62 kDa.
IP		1/40 - 1/50.

追加情報 Is unsuitable for ChIP or IHC-P.

ターゲット情報

機能 NF-kappa-B is a pleiotropic transcription factor which is present in almost all cell types and is involved in many biological processed such as inflammation, immunity, differentiation, cell growth, tumorigenesis and apoptosis. NF-kappa-B is a homo- or heterodimeric complex formed by the Rel-like domain-containing proteins RELA/p65, RELB, NFKB1/p105, NFKB1/p50, REL and NFKB2/p52. The dimers bind at kappa-B sites in the DNA of their target genes and the individual dimers have distinct preferences for different kappa-B sites that they can bind with distinguishable affinity and specificity. Different dimer combinations act as transcriptional activators or repressors, respectively. NF-kappa-B is controlled by various mechanisms of post-translational modification and subcellular compartmentalization as well as by interactions with other cofactors or corepressors. NF-kappa-B complexes are held in the cytoplasm in an inactive state complexed with members of the NF-kappa-B inhibitor (I-kappa-B) family. In a conventional activation pathway, I-kappa-B is phosphorylated by I-kappa-B kinases (IKKs) in response to different activators, subsequently degraded thus liberating the active NF-kappa-B complex which translocates to the nucleus. NF-kappa-B heterodimeric RelB-p50 and RelB-p52 complexes are transcriptional activators. RELB neither associates with DNA nor with RELA/p65 or REL. Stimulates promoter activity in the presence of NFKB2/p49.

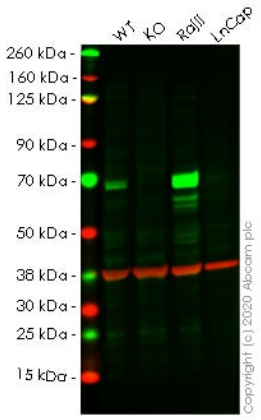
配列類似性 Contains 1 RHD (Rel-like) domain.

ドメイン Both N- and C-terminal domains are required for transcriptional activation.

翻訳後修飾 Phosphorylation at 'Thr-103' and 'Ser-573' is followed by proteasomal degradation.

細胞内局在 Nucleus. Cytoplasm > cytoskeleton > centrosome. Co-localizes with NEK6 in the centrosome.

画像



Western blot - Anti-Rel B antibody [EP614Y] (ab33907)

All lanes : Anti-Rel B antibody [EP614Y] (ab33907) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : RELB knockout HeLa cell lysate
Lane 3 : Raji cell lysate
Lane 4 : LnCap cell lysate

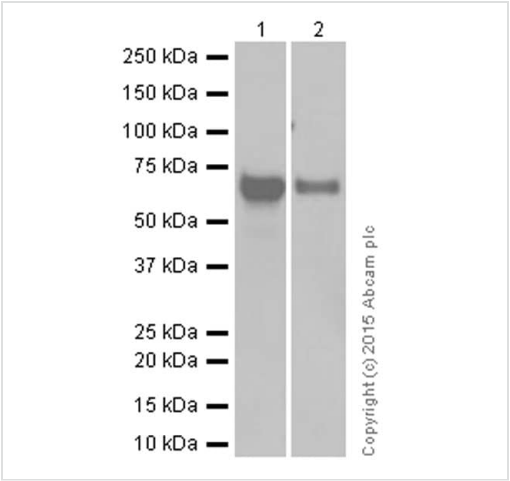
Lysates/proteins at 40 µg per lane.

Performed under reducing conditions.

Predicted band size: 62 kDa
Observed band size: 70 kDa

Lanes 1- 4: Merged signal (red and green). Green - ab33907 observed at 70 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab33907 was shown to react with Rel B in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab265948** (knockout cell lysate **ab257635**) was used. Wild-type HeLa and RELB knockout HeLa cell lysates were subjected to SDS-PAGE. ab33907 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.



Western blot - Anti-Rel B antibody [EP614Y] (ab33907)

All lanes : Anti-Rel B antibody [EP614Y] (ab33907) at 1/2000 dilution (purified)

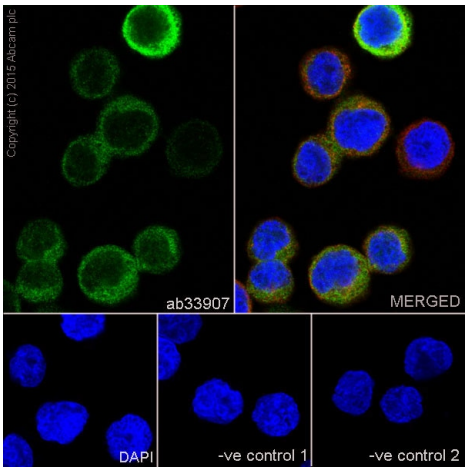
Lane 1 : Raji cell lysate
Lane 2 : Daudi cell lysate

Lysates/proteins at 20 µg per lane.

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

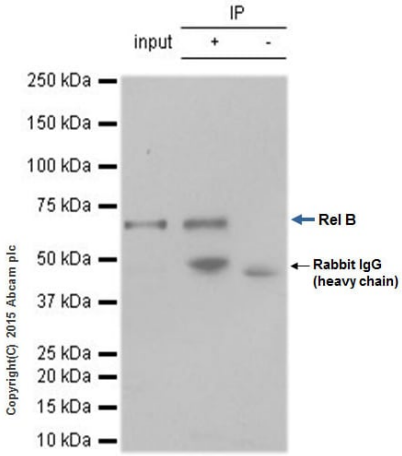
Predicted band size: 62 kDa
Observed band size: 70 kDa

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST



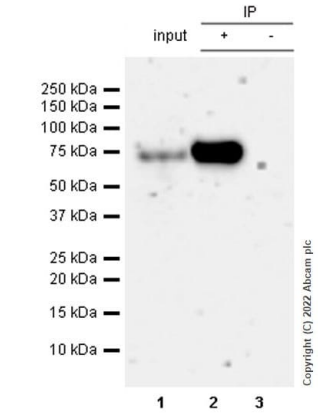
Immunocytochemistry/ Immunofluorescence - Anti-Rel B antibody [EP614Y] (ab33907)

Immunofluorescence staining of Raji cells with purified ab33907 at a working dilution of 1/100, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 488 goat anti-rabbit (**ab150077**), used at a dilution of 1/1000. **ab7291**, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with **ab150120** (Alexa Fluor® 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 4 % PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab33907 was used at a dilution of 1/500 followed by an Alexa Fluor® 594 goat anti-mouse antibody (**ab150120**) at a dilution of 1/500. For negative control 2, **ab7291** (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor® 488 goat anti-rabbit antibody (**ab150077**) at a dilution of 1/400.



Immunoprecipitation - Anti-Rel B antibody [EP614Y] (ab33907)

ab33907 (purified) at 1/20 immunoprecipitating Rel B in 10 µg Daudi cell lysate (Lanes 1 and 2, observed at 70 kDa). Lane 3 - Rabbit monoclonal IgG (**ab172730**). For western blotting, HRP Veriblot for IP (**ab131366**) was used for detection (1/1000). Blocking buffer and concentration: 5% NFDM/TBST Dilution buffer and concentration: 5% NFDM/TBST



Immunoprecipitation - Anti-Rel B antibody [EP614Y] (ab33907)

Rel B was immunoprecipitated from 0.35 mg of Raji (human Burkitt's lymphoma B lymphocyte), whole cell lysate with ab33907 at 1/30 dilution. Western blot was performed from the immunoprecipitate using 33907 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/5000 dilution.

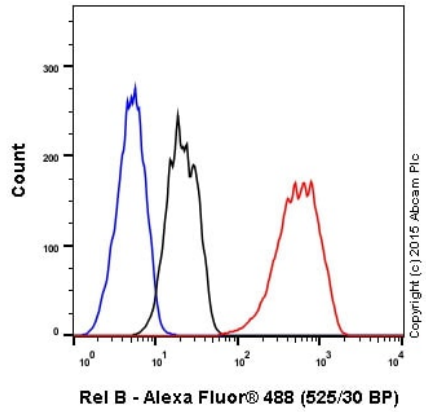
Lane 1: Raji whole cell lysate 10 µg (Input).

Lane 2: ab33907 IP in Raji whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab33907 in Raji whole cell lysate.

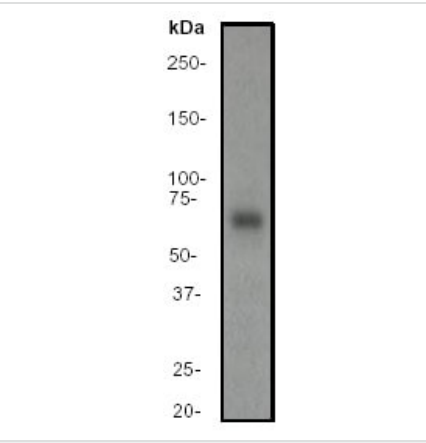
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 180 seconds.



Flow Cytometry (Intracellular) - Anti-Rel B antibody [EP614Y] (ab33907)

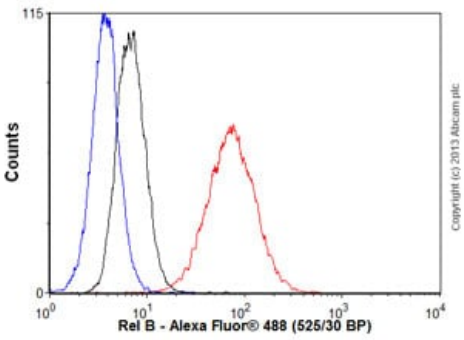
Overlay histogram showing Raji cells fixed in 80% methanol and stained with purified ab33907 at a dilution of 1/70 (red line). The secondary antibody used was Alexa Fluorr® 488 goat anti-rabbit at a dilution of 1/500. Rabbit monoclonal IgG was used as an isotype control (black line) and cells incubated in the absence of both primary and secondary antibody were used as a negative control (blue line).



Western blot - Anti-Rel B antibody [EP614Y] (ab33907)

Anti-Rel B antibody [EP614Y] (ab33907) at 1/20000 dilution (unpurified) + Raji Cell Lysate

Predicted band size: 62 kDa
Observed band size: 62 kDa



Flow Cytometry (Intracellular) - Anti-Rel B antibody [EP614Y] (ab33907)

Overlay histogram showing Raji cells stained with unpurified ab33907 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab33907, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in Raji cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

Anti-Rel B antibody [EP614Y] (ab33907)

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