

Product datasheet

Anti-Rel B antibody [EP614Y] ab33907

KO VALIDATED

Recombinant

RabMAb[®]

[3 References](#) [9 Images](#)

Overview

Product name	Anti-Rel B antibody [EP614Y]
Description	Rabbit monoclonal [EP614Y] to Rel B
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt (Intra), ICC/IF, WB, IP Unsuitable for: ChIP or IHC-P
Species reactivity	Reacts with: Human
Immunogen	Synthetic peptide within Human Rel B aa 550-650 (C terminal). The exact sequence is proprietary.
Positive control	WB: HeLa, Raji and Daudi cell lysate. IP: Daudi cell lysate, Raji whole cell lysate ICC/IF: Raji cells. Flow Cyt (intra): Raji cells.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p> <p>Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EP614Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab33907 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
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.

Application notes Is unsuitable for ChIP or IHC-P.

Target

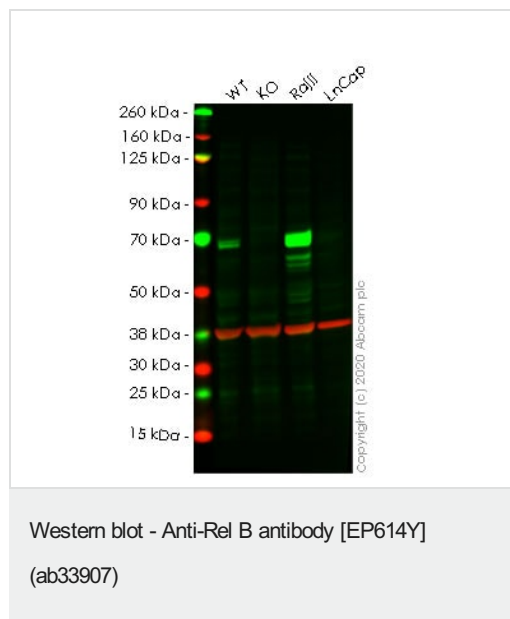
Function NF-kappa-B is a pleiotropic transcription factor which is present in almost all cell types and is involved in many biological processes such as inflammation, immunity, differentiation, cell growth, tumorigenesis and apoptosis. NF-kappa-B is a homo- or heterodimeric complex formed by the Rel-like 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.

Sequence similarities Contains 1 RHD (Rel-like) domain.

Domain Both N- and C-terminal domains are required for transcriptional activation.

Post-translational modifications Phosphorylation at 'Thr-103' and 'Ser-573' is followed by proteasomal degradation.

Cellular localization Nucleus. Cytoplasm > cytoskeleton > centrosome. Co-localizes with NEK6 in the centrosome.



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.

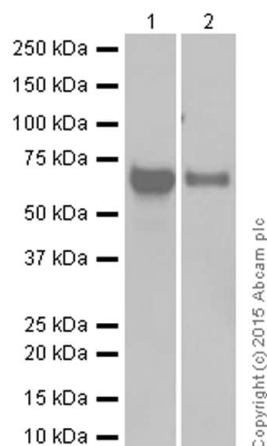
Performed under reducing conditions.

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

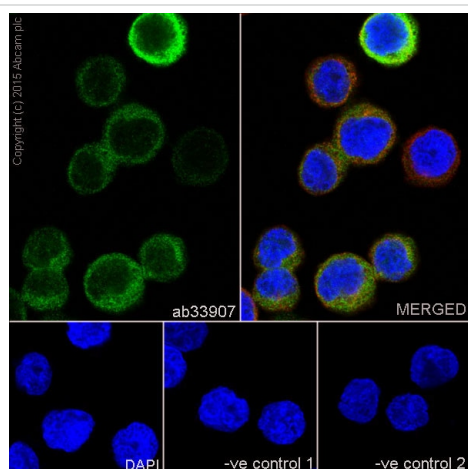
All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

Predicted band size: 62 kDa

Observed band size: 70 kDa

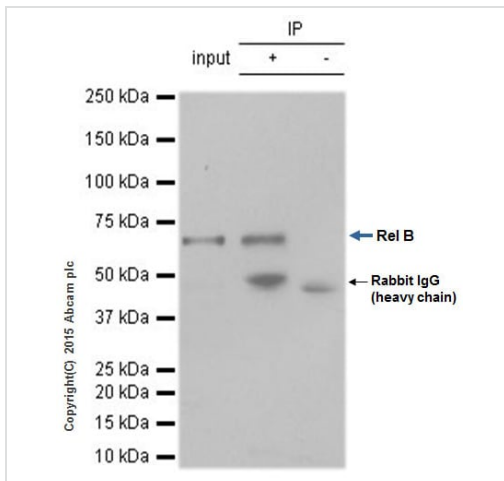
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



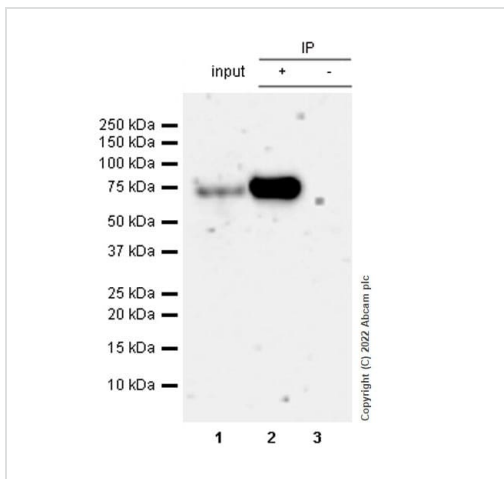
Immunocytochemistry/ Immunofluorescence - Anti-Rel B antibody [EP614Y] (ab33907)

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.

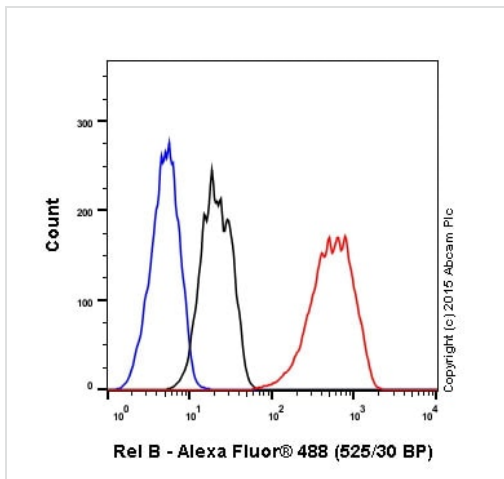
Lane 1: Raji whole cell lysate 10 µg (Input).

Lane 2: ab33907 IP in Raji whole cell lysate.

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

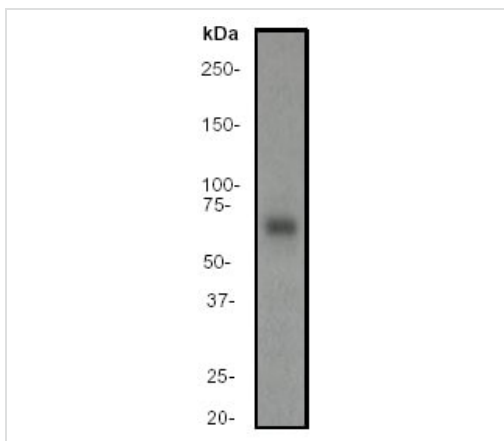
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

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 Fluor[®] 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).

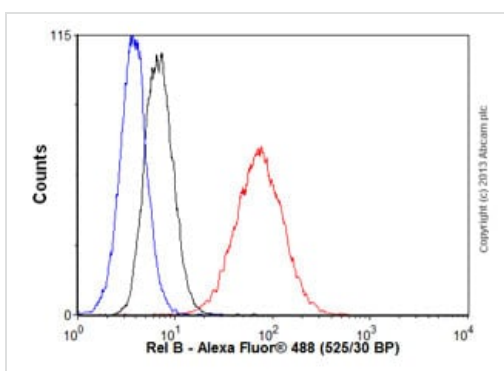


Western blot - Anti-Rel B antibody [EP614Y]
(ab33907)

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) (0.1 µg/1x10⁶ 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.

Why choose a recombinant antibody?

 <p>Research with confidence Consistent and reproducible results</p>	 <p>Long-term and scalable supply Recombinant technology</p>
 <p>Success from the first experiment Confirmed specificity</p>	 <p>Ethical standards compliant Animal-free production</p>

Anti-Rel B antibody [EP614Y] (ab33907)

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