Human/Mouse/Rat RelA/NFκB p65 Antibody

Monoclonal Mouse IgG_{2B} Clone # 532301

Catalog Number: MAB5078

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat RelA/NFκB p65 in Western blots.
Source	Monoclonal Mouse IgG_{2B} Clone # 532301
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	*E. coli*-derived recombinant human RelA/NFκB p65 isoform 1 Asn456-Ser551 Accession # Q04206
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

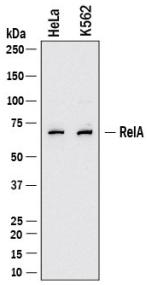
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	2 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	See Below
Intracellular Staining by Flow Cytometry	2.5 µg/10^6 cells	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Knockout Validated	RelA/NFκB p65 is specifically detected in HeLa human cervical epithelial carcinoma parental cell line but is not detectable in RelA/NFκB p65 knockout HeLa cell line.	

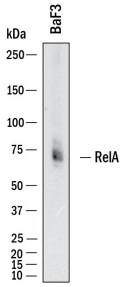
DATA

Western Blot



Detection of Human RelA/NFκB p65 by Western Blot. Western Blot shows lysates of HeLa human cervical epithelial carcinoma cell line and K562 human chronic myelogenous leukemia cell line. PVDF membrane was probed with 2 µg/ml of Mouse Anti-Human/Mouse/Rat RelA/NFκB p65 Monoclonal Antibody (Catalog # MAB5078) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for RelA/NFκB p65 at approximately 65 kDa (as indicated). This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

Western Blot



Detection of Mouse RelA/NFκB p65 by Western Blot. Western Blot shows lysates of BaF3 mouse pro-B cell line. PVDF membrane was probed with 2 µg/ml of Mouse Anti-Human/Mouse/Rat RelA/NFκB p65 Monoclonal Antibody (Catalog # MAB5078) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for RelA/NFκB p65 at approximately 65 kDa (as indicated). This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

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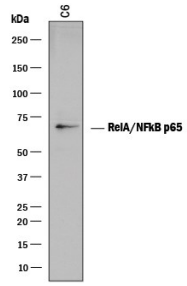
Human/Mouse/Rat RelA/NFκB p65 Antibody

Monoclonal Mouse IgG_{2B} Clone # 532301

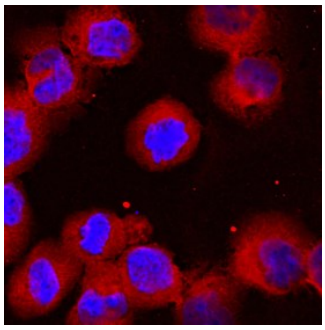
Catalog Number: MAB5078

Western Blot



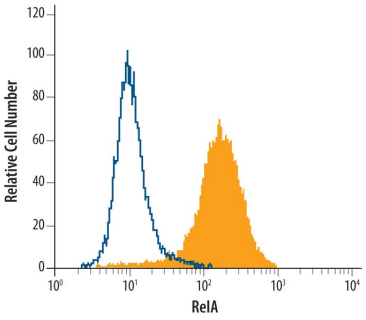
Detection of Rat RelA/NFκB p65 by Western Blot. Western blot shows lysates of C6 rat glioma cell line. PVDF membrane was probed with 2 µg/mL of Mouse Anti-Human/Mouse/Rat RelA/NF kappa B p65 Monoclonal Antibody (Catalog # MAB5078) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # Catalog # HAF018). A specific band was detected for RelA/NF kappa B p65 at approximately 65 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



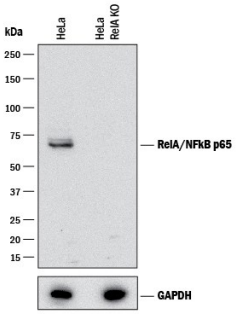
RelA/NFκB p65 in K562 Human Cell Line. RelA/NF kappa B p65 was detected in immersion fixed K562 human chronic myelogenous leukemia cell line using Mouse Anti-Human/Mouse/Rat RelA/NF kappa B p65 Monoclonal Antibody (Catalog # MAB5078) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # Catalog # NL007) and counterstained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Non-adherent Cells.

Intracellular Staining by Flow Cytometry



Detection of RelA in HeLa Human Cell Line by Flow Cytometry. HeLa human cervical epithelial carcinoma cell line was stained with Mouse Anti-Human/Mouse/Rat RelA/NF kappa B p65 Monoclonal Antibody (Catalog # MAB5078, filled histogram) or isotype control antibody (Catalog # Catalog # MAB0041, open histogram), followed by Phycoerythrin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # Catalog # F0102B). To facilitate intracellular staining, cells were fixed with paraformadehyde and permeabilized with methanol.

Knockout Validated



Western Blot Shows Human RelA/NFκB p65 Specificity by Using Knockout Cell Line. Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and RelA/NF kappa B p65 knockout HeLa cell line (KO). PVDF membrane was probed with 2 µg/mL of Mouse Anti-Human/Mouse/Rat RelA/NF kappa B p65 Monoclonal Antibody (Catalog # MAB5078) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # Catalog # HAF007). A specific band was detected for RelA/NF kappa B p65 at approximately 65 kDa (as indicated) in the parental HeLa cell line, but is not detectable in knockout HeLa cell line. GAPDH (Catalog # Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

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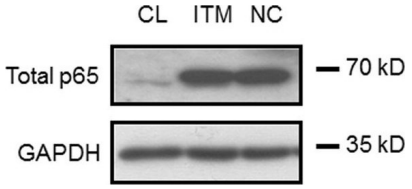
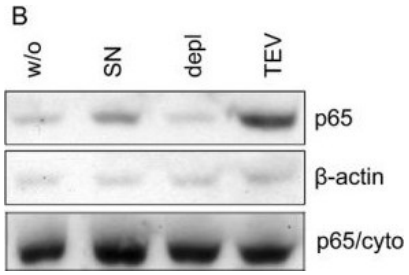
Western Blot



Detection of Human RelA/NFkB p65 by Western Blot Non-classical and intermediate monocytes express high levels of NF-κB (p65) and membrane-bound IL-1α.a Western blot analysis of total p65 and GAPDH protein levels in the three monocyte subsets. b Quantification of Western blot data shown in (a): p65 protein level was normalized to GAPDH (loading control) and expressed as a fold change with respect to CL subset. The data represent the means ± SD; n = 3. c Relative levels of phosphorylated-p65 (p-p65), measured by flow cytometry. Each line represents one donor; n = 7. d IL-1α secretion by the three monocyte subsets, measured by Luminex assay. The data represent the means ± SD; n = 3. e–f Relative expression levels of membrane-bound IL-1α (e) and cytoplasmic IL-1α (f), analyzed by flow cytometry. Each line represents one donor; n = 9. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. CL: classical, ITM: intermediate, NC: non-classical, MFI: median fluorescence intensity Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/s41419-018-0327-1), licensed under a CC-BY license. Not internally tested by R&D Systems.

Western Blot



Detection of Human RelA/NFkB p65 by Western Blot Tumor-derived EVs activate the NFκB pathway. Primary CD14+ monocytes were incubated with TEVs isolated from conditioned PCI-1 supernatants. (A) Lysates were generated at different timepoints of incubation and tested for IκB and phosphorylated P-IκB. Tubulin was used as a loading control. (B) Primary monocytes were incubated with complete PCI-1 supernatant (SN), in TEV-depleted SN (depl.) or with isolated TEV for 120 min, nuclei were isolated and the translocation of NFκB-p65 was tested with an immunoblot. Actin was used as a loading control, and the cytoplasmic fraction (p65/cyto) was used to confirm the specificity of the translocation. (C) Monocytes were incubated as in (B), and the binding capacity of NFκB p50 to the DNA was quantified with a NFκB binding assay kit. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29601673), licensed under a CC-BY license. Not internally tested by R&D Systems.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

RelA p65 (v-rel reticuloendotheliosis viral oncogene homolog A) is a 65 kDa member of the NFκB family of nuclear transcription factors. Dimers of p65 with the p50 subunit are the most common form of the NFκB transcription factor, but dimers with it or other family members can also occur. Upon activation, RelA p65 forms an heterotetramer and moves into the nucleus where it binds to specific DNA sequences. An alternatively spliced isoform that lacks amino acids (aa) 222-231 (p65Δ) does not bind DNA. Over the sequence used as an immunogen, human RelA p65 shares 96% and 98% aa identity with mouse and rat RelA p65, respectively. This portion includes one of eight potential ser/thr phosphorylation sites, two acetylation sites, and most of the Rel homology domain that interacts with IκB inhibitors.

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