

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human and mouse RelA/NFκB p65 in Western blots.
Source	Monoclonal Mouse IgG _{2B} Clone # 532301
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	<i>E. coli</i> -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.

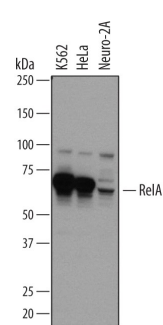
APPLICATIONS

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.	

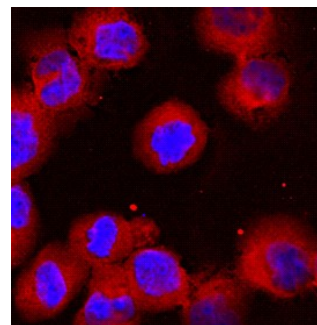
DATA

Western Blot



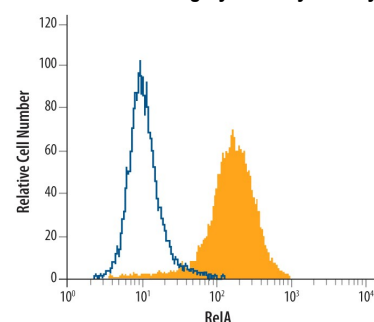
Detection of Human and Mouse RelA/NFκB p65 by Western Blot. Western blot shows lysates of K562 human chronic myelogenous leukemia cell line, HeLa human cervical epithelial carcinoma cell line, and Neuro-2A mouse neuroblastoma cell line. PVDF membrane was probed with 2 μg/mL of Mouse Anti-Human/Mouse RelA/NFκB p65 Monoclonal Antibody (Catalog # MAB5078) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for RelA/NFκB p65 at approximately 70 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



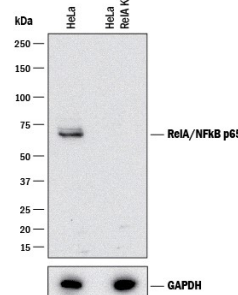
RelA/NFκB p65 in K562 Human Cell Line. RelA/NFκB p65 was detected in immersion fixed K562 human chronic myelogenous leukemia cell line using Mouse Anti-Human/Mouse RelA/NFκB p65 Monoclonal Antibody (Catalog # MAB5078) at 10 μg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # 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 RelA/NFκB p65 Monoclonal Antibody (Catalog # MAB5078, filled histogram) or isotype control antibody (Catalog # MAB0041, open histogram), followed by Phycoerythrin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0102B). To facilitate intracellular staining, cells were fixed with paraformaldehyde 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/NFκB p65 knockout HeLa cell line (KO). PVDF membrane was probed with 2 μg/mL of Mouse Anti-Human/Mouse RelA/NFκB p65 Monoclonal Antibody (Catalog # MAB5078) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for RelA/NFκB p65 at approximately 65 kDa (as indicated) in the parental HeLa cell line, but is not detectable in knockout HeLa cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none">● 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 a 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.