

DATASHEET

NF-kappaB p65 (Phospho-T254) Rabbit Polyclonal Antibody

CAT. NO. APA09489

KEY FEATURES

Target	NF-kappaB p65 (Phospho-T254)	Source / Host	Rabbit
Reactivity	Human, Mouse, Rat, Bovine, Monkey, Pig	Clonality	Polyclonal
Applications	WB, IHC, IF/ICC, IP	Conjugation	Unconjugated
Form / Buffer	Liquid in 0.42% Potassium phosphate, 0.87% Sodium chloride, pH 7.3, 30% glycerol, and 0.01% sodium azide.	Storage	at-20°C

BACKGROUND

NF-kappa-B is a pleiotropic transcription factor present in almost all cell types and is the endpoint of a series of signal transduction events that are initiated by a vast array of stimuli related to 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 heterodimeric RELA-NFKB1 complex appears to be most abundant one. 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.

APPLICATION

To ensure optimal assay performance, AREX recommends conducting reagent titration tailored to each testing system for optimal detection results.

WB	1:500 - 1:1000
IHC	1:100 - 1:200
IF/ICC	1:50 - 1:200
IP	1:10 - 1:100

*Results are sample-specific. Please refer to your local assay conditions and test parameters for reference.

PRODUCT OVERVIEW

Description	Rabbit polyclonal antibody to NF-kappaB p65 (Phospho-T254)
Specificity	Recognizes endogenous levels of NF-kappaB p65 protein only when phosphorylated at T254.
Antibody Type	Primary antibody
Immunogen	KLH-conjugated synthetic phosphopeptide corresponding to residues surrounding T254 of human NF-kappaB p65 protein. The exact sequence is proprietary.
Purification	The antibody was purified by immunogen affinity chromatography.
Molecular Weight	Predicted: 60 kD; Observed: 65 kD
Form/Buffer	Liquid in 0.42% Potassium phosphate, 0.87% Sodium chloride, pH 7.3, 30% glycerol, and 0.01% sodium azide.
Alternative Names	NFKB3; Transcription factor p65; Nuclear factor NF-kappa-B p65 subunit; Nuclear factor of kappa light polypeptide gene enhancer in B-cells 3
Gene Symbol	RELA
Entrez Gene	5970(Human); 19697(Mouse)
SwissProt	Q04206(Human); Q04207(Mouse)

*AREX continuously optimizes our products. Webpage content may not reflect the latest updates. For inquiries, please contact info@arexbio.com or your local distributor.

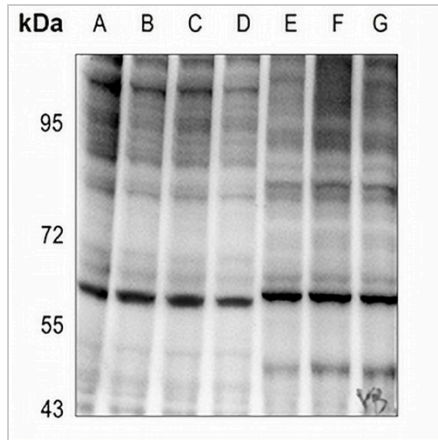
*Clone Number, Reactivity, Source/Host and Clonality can be found in the product name and Key Features section above.

DATASHEET

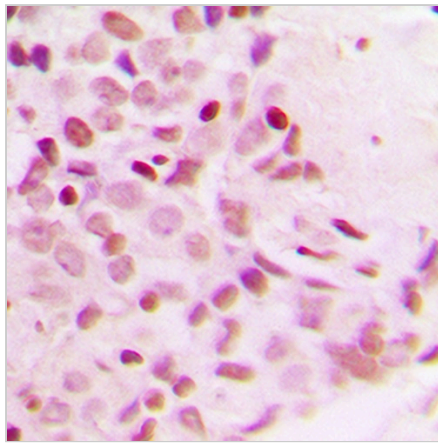
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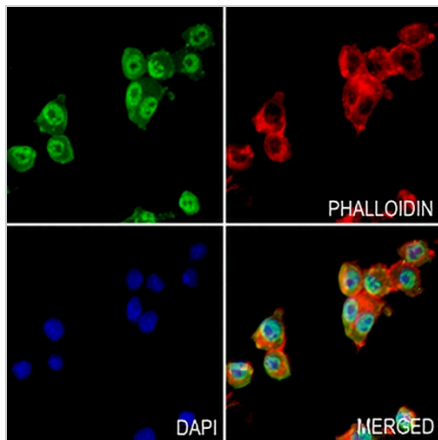
DATA



Western blot analysis of NF-kappaB p65 (Phospho-T254) expression in MCF7-LPS-30mins (A), MCF7-LPS-15mins (B), MCF7-LPS-5mins (C), MCF7 (D), HeLa-TNFa-15mins (E), HeLa-TNFa-5mins (F), HeLa (G) whole cell lysates. (Predicted band size: 60 kD; Observed band size: 65 kD)



Immunohistochemical analysis of NF-kappaB p65 (Phospho-T254) staining in human breast cancer formalin fixed paraffin embedded tissue section. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0). The section was then incubated with the antibody at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.



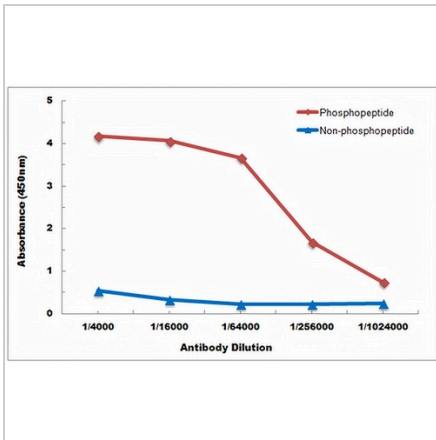
Immunofluorescent analysis of NF-kappaB p65 (Phospho-T254) staining in BV2 cells. Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with the primary antibody in 3% BSA-PBS and incubated overnight at 4 °C in a humidified chamber. Cells were washed with PBST and incubated with a AREX® Fluor 488 - conjugated secondary antibody (green) in PBS at room temperature in the dark. Phalloidin - AREX® Fluor 594 was used to stain Actin filaments (red). DAPI was used to stain the cell nuclei (blue).

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DATA (CONTINUED)



Direct ELISA antibody dose-response curve using Anti-NF-kappaB p65 (Phospho-T254) Antibody. Antigen (Phosphopeptide and non-phosphopeptide) concentration is 5 ug/ml. Goat Anti-Rabbit IgG (H&L) - HRP was used as the secondary antibody, and signal was developed by TMB substrate.

STORAGE

Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles.

NOTE

For Research Use Only. Not for diagnostic, therapeutics, prophylactic or in vivo use.