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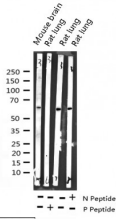
## Phospho-NF kappaB p65 (Thr254) Ab

Cat.#: AF3391  
Size: 100ul,200ul

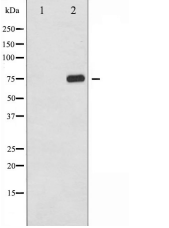
Concn.: 1mg/ml  
Source: Rabbit

Mol.Wt.: 65kDa  
Clonality: Polyclonal

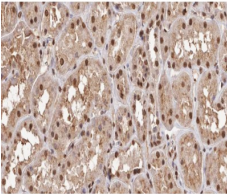
Application:	WB 1:500-1:2000 IHC 1:50-1:200 IP, IF/ICC 1:100-1:500
Reactivity:	Human,Mouse,Rat
Purification:	The Ab is from purified rabbit serum by affinity purification via sequential chromatography on phospho- and non-phospho-peptide affinity columns.
Specificity:	Phospho-NF- kappaB p65 (Thr254) Ab detects endogenous levels of NF- kappaB p65 only when phosphorylated at Threonine 254.
Immunogen:	A synthesized peptide derived from human NF- kappaB p65 around the phosphorylation site of Threonine 254.
Uniprot:	Q04206
Description:	NFKB1 (MIM 164011) or NFKB2 (MIM 164012) is bound to REL (MIM 164910), RELA, or RELB (MIM 604758) to form the NFKB complex. The p50 (NFKB1)/p65 (RELA) heterodimer is the most abundant form of NFKB. The NFKB complex is inhibited by I-kappa-B proteins (NFKBIA, MIM 164008 or NFKBIB, MIM 604495), which inactivate NFKB by trapping it in the cytoplasm.
Subcellular Location:	Nucleus. Cytoplasm. Nuclear, but also found in the cytoplasm in an inactive form complexed to an inhibitor (I-kappa-B). Colocalized with RELA in the nucleus upon TNF-alpha induction.
Similarity:	the 9aaTAD motif is a transactivation domain present in a large number of yeast and animal transcription factors.
Storage Condition and Buffer:	Rabbit IgG in phosphate buffered saline , pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol.Store at -20 °C.Stable for 12 months from date of receipt.



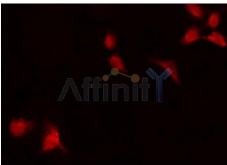
Western blot analysis of Phospho-NF kappaB p65 (Thr254) expression in various lysates



Western blot analysis of NF- kappaB p65 phosphorylation expression in TNF- $\alpha$  treated 293 whole cell lysates, The lane on the left is treated with the antigen-specific peptide.



AF3391 at 1/200 staining human kidney tissue sections by IHC-P. The tissue was formaldehyde fixed and a heat mediated antigen retrieval step in citrate buffer was performed. The tissue was then blocked and incubated with the Ab for 1.5 hours at 22°C. An HRP conjugated goat anti-rabbit Ab was used as the secondary.



AF3391 staining HeLa by IF/ICC. The sample were fixed with PFA and permeabilized in 0.1% Triton X-100, then blocked in 10% serum for 45 minutes at 25°C. The primary Ab was diluted at 1/200 and incubated with the sample for 1 hour at 37°C. An Alexa Fluor 594 conjugated goat anti-rabbit IgG (H+L) Ab, diluted at 1/600, was used as the secondary Ab.

**IMPORTANT:** For western blot, incubate membrane with diluted primary Ab in 5% w/v milk, 1X TBS, 0.1% Tween@20 at 4°C with gentle shaking, overnight.

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