

**Datasheet for 600-401-267****IKK E phospho T501 Antibody****Overview**

<b>Description:</b>	Anti-IKKe pT501 (RABBIT) Antibody - 600-401-267
<b>Item No.:</b>	600-401-267
<b>Size:</b>	100 µg
<b>Applications:</b>	ELISA, WB, IHC
<b>Reactivity:</b>	Human
<b>Host Species:</b>	Rabbit

**Product Details**

<b>Background:</b>	Nuclear Factor kappa B (NF-κB) is a ubiquitous transcription factor and an essential mediator of gene expression during the activation of immune and inflammatory responses. NF-κB mediates the expression of a great variety of genes in response to extracellular stimuli. NF-κB is associated with IκB proteins in the cytoplasm of the cell, which inhibit NF-κB activity. IκB proteins are phosphorylated by an IκB kinase complex consisting of at least three proteins, IKKα, IKKβ, and IKKγ. Isolated from a cDNA library of LPS-stimulated mouse macrophage cells, a novel molecule in the IKK complex has been recently identified and designated IKKi and/or IKKe. IKKe is required for the activation of NF-κB by mitogens and T cell receptors but not by TNFα or IL-1. LPS increases the IKKe mRNA level in mouse macrophage cell lines. This protein has significant sequence homology with kinase domains of IKKα and IKKβ. Overexpression of wild type IKKe in cells phosphorylates Ser32 and Ser36 of IκBα. Anti-IKKe pT501 antibody is ideal for investigators involved in NFκB and apoptosis research.
<b>Synonyms:</b>	rabbit anti-IKK epsilon pT501 antibody, rabbit anti-IKKE pT501 antibody, I kappa B kinase epsilon antibody, IKK-epsilon, IKK-E, Inducible I kappa-B kinase, IκBKE antibody, IκBKE protein antibody, Inhibitor of nuclear factor kappa B kinase subunit epsilon antibody, IKKE, IKKI
<b>Host Species:</b>	Rabbit
<b>Clonality:</b>	Polyclonal
<b>Format:</b>	IgG

**Target Details**

<b>Gene Name:</b>	IKBKE
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<b>Reactivity:</b>	Human
<b>PTM Specificity:</b>	Phosphorylation
<b>Immunogen Type:</b>	Conjugated Peptide
<b>Immunogen:</b>	IKKe phospho peptide corresponding to a region of the human protein surrounding pT501 conjugated to KLH.
<b>Purity/Specificity:</b>	Anti-IKKe pT501 antibody was affinity purified from monospecific antiserum by immunoaffinity purification against the phosphopeptide and cross adsorption against the non-phosphorylated form of the peptide followed by non-adsorption against a non-specific peptide backbone to further reduce non-specific reactivity. This phospho specific polyclonal antibody is specific for phosphorylated pT501 human IKKe. Reactivity with non-phosphorylated IKKe is minimal. Cross reactivity with pT501 phosphorylated IKKe from mouse, rat or other species has not been determined.
<b>Relevant Links:</b>	<ul style="list-style-type: none"><li>• <a href="#">NCBI - Q9ROT8.1</a></li><li>• <a href="#">NCBI - Q14164.1</a></li><li>• <a href="#">UniProtKB - Q14164</a></li><li>• <a href="#">GenelD - 9641</a></li></ul>

## Application Details

<b>Tested Applications:</b>	ELISA, WB
<b>Suggested Applications:</b>	IHC (Based on references)
<b>Application Note:</b>	IKKe pT501 antibody is tested in ELISA, western blotting, and although not tested, this antibody is likely functional in immunohistochemistry and immunoprecipitation. An 85 kDa band corresponding to human IKKe is detected. HeLa cells or TNF inducible KBM-5 cells can be used as a positive control. Researchers should determine optimal titers for other applications.
<b>Assay Dilutions:</b>	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
<b>ELISA:</b>	1:5,000 - 1:25,000
<b>WB:</b>	1:500 - 1:3,000

## Formulation

<b>Physical State:</b>	Liquid (sterile filtered)
<b>Concentration:</b>	1 mg/mL by UV absorbance at 280 nm
<b>Buffer:</b>	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

**Preservative:** 0.1% (w/v) Sodium Azide

**Stabilizer:** None

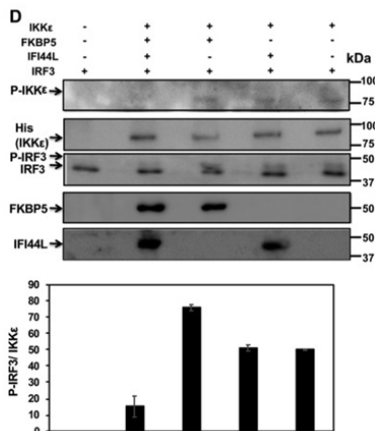
## Shipping & Handling

**Shipping Condition:** Dry Ice

**Storage Condition:** Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.

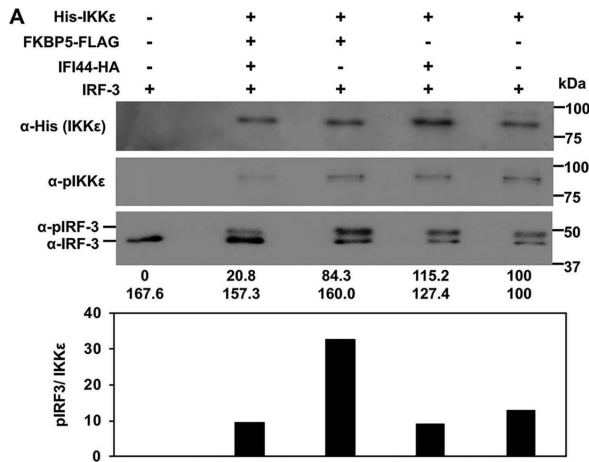
**Expiration:** Expiration date is one (1) year from date of receipt.

## Images



### Western Blot

(D) Human 293T cells were silenced for IFI44 or FKBP5 and were transfected with plasmids expressing His-IKKε, IFI44L-HA, and FKBP5-FLAG. At 24 hpt, IKKε complexes were purified with an anti-His antibody and assayed in a kinase assay using recombinant human IRF-3 as the substrate. Levels of total and phosphorylated forms of IRF-3 were analyzed by Western blotting using specific antibodies. Levels of IKKε were determined using an anti-His-specific antibody and levels of phospho-IKKε were evaluated using an anti-p-IKKε antibody by Western blotting. Levels of IFI44L-HA and FKBP5-FLAG proteins were detected with anti-HA-specific and anti-FLAG-specific antibodies, respectively. Protein bands were quantified by densitometry using ImageJ software (v1.46), and levels of pIRF-3 were normalized to the levels of IKKε (bottom graphic). The results show error bars and means of results from two independent experiments. Molecular weight markers (in kilodaltons) are indicated on the right. Fig 8. PMID: 31434731



### Western Blot

IFI44 decreases the kinase activity of IKK $\beta$  and IKK $\epsilon$ . Human 293T cells were silenced for IFI44, or for FKBP5, and were transfected with plasmids expressing His-IKK $\epsilon$  (A) or MYC-IKK $\beta$  (B), together with IFI44-HA, and FKBP5-FLAG expression plasmids. At 24 hpt, IKK $\epsilon$  (A) and IKK $\beta$  (B) complexes were purified with anti-His and anti-MYC antibodies, respectively, and these complexes were assayed in kinase assays using IRF-3 (for the IKK $\epsilon$  complexes shown in panel A) and I $\kappa$ B $\alpha$  (for the IKK $\beta$  complexes shown in panel B) as substrates. The levels of phosphorylated and unphosphorylated forms of IRF-3 (panel A, bottom blot) and I $\kappa$ B $\alpha$  (panel B, third and fourth blots) were analyzed by Western blotting using specific antibodies. Levels of IKK $\epsilon$  were analyzed using an anti-His-specific antibody (A, first blot) and anti-pIKK $\epsilon$  (A, second blot), and levels of IKK $\beta$  were analyzed using an anti-MYC-specific antibody (B, first blot) and anti-pIKK $\beta$  (B, second blot). Western blots were quantified by densitometry using ImageJ software (v1.46). Protein expression levels in cells expressing IKK $\epsilon$  (A) and IKK $\beta$  (B) alone were assigned a value of 100% for comparisons with the levels of expression in cells expressing the different combinations of IKK $\epsilon$ /IFI44/FKBP5 (A) or IKK $\beta$ /IFI44/FKBP5 (B) (numbers are indicated below each plot). pIRF-3 and IRF-3 levels (observed in the same bottom blot in panel A) and pI $\kappa$ B $\alpha$  and I $\kappa$ B $\alpha$  (third and bottom blot in panel B) are represented with numbers below each blot. Levels of pIRF-3 and pI $\kappa$ B $\alpha$  normalized to the levels of IKK $\epsilon$  and IKK $\beta$  are represented in the bottom graphs in panels A and B, respectively. Molecular weight markers are indicated (in kilodaltons) on the right. Figure provided by CiteAb. Source: MBio, PMID: 31455651.

## References

- DeDiego ML et al. Interferon-induced protein 44 interacts with cellular FK506-binding protein 5, negatively regulates host antiviral responses, and supports virus replication. *mBio*. (2019)
- DeDiego ML et al. Novel functions of IFI44L as a feedback regulator of host antiviral responses. *J Virol*. (2019)
- Sweeney SE, Mo L, Firestein GS. Antiviral gene expression in rheumatoid arthritis: role of IKKepsilon and interferon regulatory factor 3. *Arthritis Rheum*. (2007)

## Disclaimer

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