Recombinant IKKε protein

Catalog No: 81117, 81817
Lot No: 01118001
Expressed In: Baculovirus

Quantity: 20, 1000 µg
Concentration: 0.3 µg/µl
Source: Human

Buffer Contents: Recombinant IKKε protein is supplied at a concentration of 0.3 µg/µl in 25 mM HEPES-NaOH pH 7.5, 300 mM NaCl, 10% glycerol, 0.04% Triton X-100 and 0.5 mM TCEP.

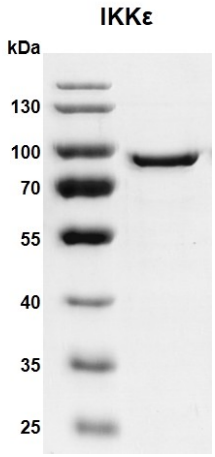
Background: IKKε (Inhibitor Of Nuclear Factor Kappa B Kinase Subunit Epsilon, also known as IKBKE), is a noncanonical I-kappa-B kinase (IKK) that is essential for regulating antiviral signaling pathways. It's a serine/threonine kinase that plays an essential role in regulating inflammatory responses to viral infection, through the activation of the type I IFN, NF-kappa-B and STAT signaling. It also involves in TNFα and inflammatory cytokines, like Interleukin-1, signaling. Following activation of viral RNA sensors, such as RIG-I-like receptors, IKKε associates with DDX3X and phosphorylates interferon regulatory factors (IRFs), IRF3 and IRF7, as well as DDX3X. This activity allows subsequent homodimerization and nuclear translocation of the IRF3 leading to transcriptional activation of pro-inflammatory and antiviral genes including IFNβ. In order to establish such an antiviral state, IKKε forms several different complexes whose composition depends on the type of cell and cellular stimuli. Thus, several scaffolding molecules including IPS1/MAVS, TANK, AZI2/NAP1 or TBKBP1/SINTBAD can be recruited to the IKKε-containing-complexes. It can be activated by polyubiquitination in response to TNF&alpha and interleukin-1, regulate the NF-kappa-B signaling pathway through, at least, the phosphorylation of CYLD. It can phosphorylate inhibitors of NF-kappa-B thus leading to the dissociation of the inhibitor/NF-kappa-B complex and ultimately the degradation of the inhibitor. In addition, IKKε is also required for the induction of a subset of ISGs which displays antiviral activity, may be through the phosphorylation of STAT1 at Ser-708. Phosphorylation of STAT1 at Ser- 708 seems also to promote the assembly and DNA binding of ISGF3 (STAT1:STAT2:IRF9) complexes compared to GAF (STAT1: STAT1) complexes, in this way regulating the balance between type I and type II IFN responses.

Protein Details: Recombinant human IKKε protein was expressed in a baculovirus expression system as the full length protein (accession number NP_054721.1) with a N-terminal FLAG tag. The molecular weight of the protein is 81.7 kDa.

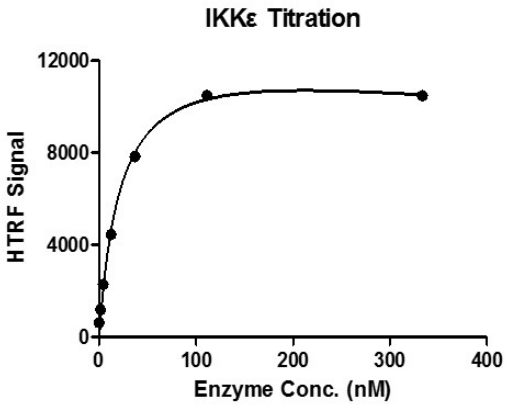
Application Notes: This protein is suitable for use in the study of enzyme kinetics, inhibitor screening, and selectivity profiling.

Kinase Activity Assay Conditions: 1 µM STK S3 substrate was incubated with different concentrations of IKKε protein in 10 µl reaction system containing 1×Enzymatic Buffer, 5 mM MgCl2, 1 mM DTT and 100 µM ATP for 1 hour. The 10 µl detection reagents containing STK antibody and SA-XL665, each of which was 1:100 diluted with 1× Detection Buffer, were added and incubated with the reactions for 30 min. All the operations and reactions were performed at room temperature, and HTRF KinASE STK assay was used to detect the enzymatic activity.

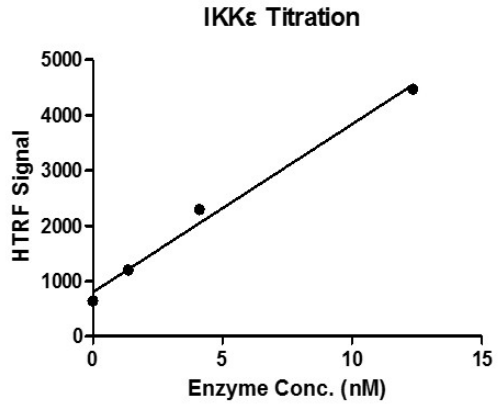
Storage and Guarantee: Recombinant proteins in solution are temperature sensitive and must be stored at -80°C to prevent degradation. Avoid repeated freeze/thaw cycles and keep on ice when not in storage. This product is for research use only and is not for use in diagnostic procedures. This product is guaranteed for 6 months from date of arrival.



Recombinant IKKε protein
10% SDS-PAGE Coomassie staining
MW: 81.7 kDa
Purity: >90%



HTRF assay for IKKε activity
1 µM STK S3 substrate was incubated with different concentrations of IKKε protein in reaction system for 1 hour. The detection reagents were added and incubated with the reactions for 30 min. All the operations and reactions were performed at room temperature, and HTRF KinASE TK assay was used to detect the enzymatic activity.



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