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Certificate of Analysis

IKKγ

(Recombinant protein expressed in Sf21 insect cells)
Item # 23-060, 23-060-K, 23-060M
Parent Lot # D13AP004N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: *N*-terminal cMyc and GST-tagged, recombinant human IKKγ full length, expressed by baculovirus in Sf21 insect cells. Purified using glutathione sepharose.

Purity 81.2% by SDS-PAGE and Coomassie blue staining. MW = 76kDa.

Formulation: 0.975mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

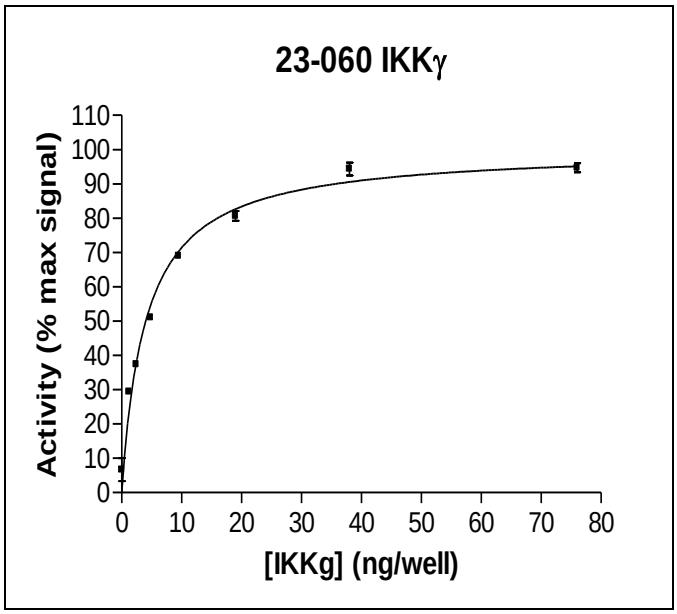
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

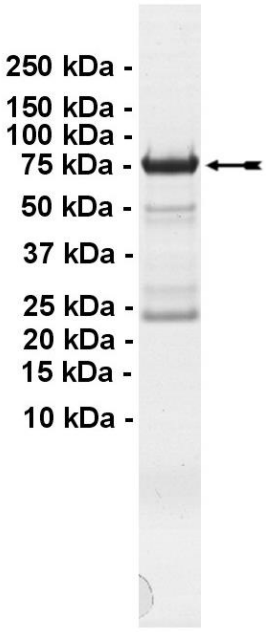
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Assay: This enzyme was titrated in a ubiquitination assay and the results normalised against the maximum signal.



Protein Identity: Confirmed identity as IKKγ by mass spectrometry.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of IKKγ.

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Assay Protocol

Reagents:

1. **UBE1, active (Item # 23-021)**
2. **UbcH5a, active (Item # 23-029)**
3. **TRAF6, active (Item # 23-059)**
4. **IKKγ (Item # 23-060)**
5. **1x Reaction Buffer**
6. **Biotinylated-Ubiquitin**
7. **Stop Solution**

Assay Outline:

All enzymes and reagents are diluted in the 1x reaction buffer (25mM MOPS pH7.5, 0.01% Tween 20, 5mM $MgCl_2$).

IKKγ is incubated with 25mM MOPS pH7.5, 0.01% Tween 20, 5mM $MgCl_2$, 10µM ATP, 10nM UBE1, 1000nM UbcH5a, 0.05ug TRAF6, and 2µM biotinylated-ubiquitin. The reaction is initiated with the addition of biotinylated-ubiquitin. After 30 minutes at room temperature the reaction is terminated by the addition of 25mM MOPS pH7.5 containing 125mM EDTA, 150mM NaCl, and 0.05% Tween 20. Reaction products are separated by capture onto a microplate coated with anti-c-Myc antibody and washing with PBS containing 0.05% Tween 20. Ubiquitination of IKKγ is measured by detection of bound ubiquitin via electrochemiluminescence.

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IKKγ Information

Protein human IKKγ

Accession number GenBank AF091453

Alternative Names NF-kappa-B essential modulator. Inhibitor of nuclear factor kappa-B kinase subunit gamma, FIP-3, NEMO

Key Facts IKKγ has been shown to play a critical role in NF-κB activation, as the regulatory subunit of IκB kinase, which phosphorylates inhibitors of NF-κB leading to their degradation. Upon cell stimulation, IKKγ can be modified by Lys-63-linked or linear polyubiquitination, both of which are suggested to play key roles in cell signalling, including NF-κB activation. One of the E3 ligases responsible for Lys-63-linked IKKγ polyubiquitination is TRAF6, which participates in several signalling pathways controlling immunity, skin development, osteoclast formation and brain functions.

Related Products UBE1, active (Item # 23-021), UbcH5a, active (Item # 23-029), TRAF6, active (Item 23-059)

Selected References

Rothwarf D. M. *et al*., IKKγ is an essential regulatory subunit of the IκB kinase complex. Nature, *395:* 297-300, 1998

Wu C-J. *et al*., Sensing of Lys 63-linked polyubiquitination by NEMO is a key event in NF-κB activation. Nature Cell Biology, *8:* 398-406, 2006

Hadian K. *et al.,* NF-κB Essential Modulator (NEMO) Interaction with Linear and Lys-63 Ubiquitin Chains Contributes to NF-κB Activation. J. Biol. Chem., *286*: 26107-26117, 2011

Wu H. *et al*., Structural studies of NEMO and TRAF6: implications in NF-κB activation. Adv Exp Med Biol. *691*: 89-91, 2011

Reviewed and approved by site quality representative.