Product Datasheet

IKK gamma Inhibitor Peptide Set
NBP2-26504

Unit Size: 2 mg

Store at -20C. Avoid freeze-thaw cycles.

Publications: 21

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# IKK gamma Inhibitor Peptide Set

## Product Information

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<th><strong>Unit Size</strong></th>
<th>2 mg</th>
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<tbody>
<tr>
<td><strong>Concentration</strong></td>
<td>Lyoph</td>
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<tr>
<td><strong>Storage</strong></td>
<td>Store at -20C. Avoid freeze-thaw cycles.</td>
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<tr>
<td><strong>Reconstitution Instructions</strong></td>
<td>Please contact technical support for detailed reconstitution instructions.</td>
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## Product Description

### Gene ID
- **Gene ID**: 8517
- **Gene Symbol**: IKBKG
- **Species**: Human, Mouse, Rat

### Specificity/Sensitivity
- IKK-Gamma NEMO Binding Domain (NBD) inhibitor peptide contains a protein transduction (PTD) sequence (DRQIKIWFQNRRMKWKK) derived from antennapedia which renders the peptide cell permeable.
- The control peptide consists of only the PTD sequence.

### Immunogen
- Functions as an IKK-Alpha/IKK-Beta decoy by binding to IKK-Gamma NBD, thereby preventing formation of the IKK complex.

### Notes
- The IKK-gamma inhibitory peptide contains a protein transduction (PTD) sequence (DRQIKIWFQNRRMKWKK) derived from antennapedia which renders the peptide cell permeable (Derossi et al, The third helix of the antennapedia homeodomain translocates through biological membranes. J Biol Chem. 269:10444-10450 (1994)). The control peptide consists of only the PTD sequence.

### Inhibitor Family
- NFkB

### Inhibitor Target
- NBD

### Inhibitor Content
1. IKK-gamma NEMO Binding Domain (NBD) Inhibitor Peptide: 2 x 1 mg (lyophilized) DRQIKIWFQNRRMKWKKTALDWSWLQTE (IKK-gamma/NEMO binding sequence is underlined). Molecular weight: 3692
2. Antennapedia Control peptide: 2 x 1 mg (lyophilized) DRQIKIWFQNRRMKWKK. Molecular weight: 2361

## Product Application Details

### Applications
- Functional (Inhibition), In vitro assay, In vivo assay

### Recommended Dilutions
- In vitro assay, In vivo assay, Functional (Inhibition)
Inhibition of NF-κB activity in vivo and in vitro by interfering with IKK complex formation

Researchers can study the effect of NBD inhibitor peptide using a variety of methods. Quantitative readout assays include NF-κB/p65 ActivELISA Kit, Phospho-IκBα ActivELISA Kit and EMSA. Immunocytochemistry can also be used as a readout assay for visualizing the subcellular localization of NF-κB; activated NF-κB localizes to the nucleus, whereas NF-κB in the cytoplasm is generally considered inactive.

U266 cells and EMSA assay are used to quality control every lot of the NBD inhibitor peptide set (Fig. 2). This protocol is written for U266, a human multiple myeloma cell line. Multiple myeloma is a B-cell malignancy, and a number of multiple myeloma cell lines, including U266, have been found to have constitutively active NF-κB. The EMSA assay shows that NBD suppressed the constitutive activation of NF-κB in U266 cells. The immunocytochemistry data provides supporting evidence that the nuclear translocation of NF-κB was lost when the U266 cells were incubated with NBD (Fig. 3).

Researchers must optimize assay methods for the NBD inhibitory peptide for different cell types. These include incubation time and amount of peptide used in an experiment. Depending on the cell types, morphology of cells may change after 2 hr of incubation with NBD peptide. For example, CHO cells become rounder in appearance after 2 hr incubation with NBD peptides. Since NF-κB is an important molecule for cell survival and proliferation, blockade of NF-κB activation by inhibiting IKK complex formation may prevent cell proliferation, which has been observed for CHO cells at 12 hrs. Researchers are advised to monitor the viability of cells for long-term incubation with the inhibitor. Use in Functional inhibition reported in scientific literature (PMID 24361600)
In vivo assay: IKK gamma Inhibitor Peptide Set [NBP2-26504] - NBD peptide blocks constitutive NF-kB as shown by EMSA. U266 cells were treated with 100 uM of control or NBD peptide for different time periods. Nuclear extracts were isolated and checked for NF-kB-DNA binding activity.

In vivo assay: IKK gamma Inhibitor Peptide Set [NBP2-26504] - NBD peptide blocks constitutive NF-kB activation in human multiple myeloma cells. U266 cells were treated with 100 uM of control (A & B) or NBD peptide (C & D) for 12 hr, cytopspun, plated on glass slides, air dried for 1 hr at room temperature and fixed with cold acetone. Slides were blocked with 5% normal goat serum for 1 hr and then incubated with rabbit polyclonal anti-human p65 antibody (A & C) followed by Ig-Alexa 594 second step. In control peptide treated cells, p65 translocates to nucleus (A), whereas NBD peptide prevents translocation of p65 into the nucleus (C). B & D: Nuclear staining with DNA binding dye.

IKK gamma Inhibitor Peptide Set [NBP2-26504] - The IKK complex consists of IKKalpha + IKKbeta+ NEMO. NEMO (IKKgamma/NF-kB Essential MOdulator) is a scaffold protein required for the kinase activity of IKKalpha + IKKbeta. NBD (NEMO Binding Domain) inhibits formation of the IKK complex (IKKalpha + IKKbeta+ NEMO) by binding to NEMO and blocking IKK complex formation. In this regard, NBD is considered to be an 'IKKalpha and IKKbeta decoy' Blocking IKK complex formation suppresses downstream events in the NF-kB signaling pathway that rely on the IKK complex including phosphorylation of IkBalpha, and subsequence IkBalpha ubiquination, degradation and release of NF-kB. Hence, NF-kB is retained in the cytoplasm in its inactive state.

Functional (Inhibition): IKK gamma Inhibitor Peptide Set [NBP2-26504] - TLR5/NF-kB/SEAPorter HEK 293 (NBP2-26277) cells were plated in 96-well plates at 5 x 10^4 cells/well for 16 h. Cells were preincubated with different concentrations (0, 1, 5, 10, 25 and 50 uM) of Inhibitory Peptide (NBP2-26504) and Control Peptide (NBP2-29334) for 1 h. Cells were then stimulated with 1 ng/ml Flagellin (NBP2-25289) for 24 h. Secreted alkaline phosphatase (SEAP) was analyzed using SEAPorter Assay Kit (NBP2-25285). *p < 0.05 versus control peptide at the corresponding concentrations (Mann-Whitney U test).
Publications


Esposito E, Napolitano G, Pescatore A et al. COMMD7 as a novel NEMO interacting protein involved in the termination of NF-kBsignaling J. Cell. Physiol. 2015 Jun 08 [PMID: 26060140] (Func, Mouse)


Mirzaei Siroos, Guerchaft Michel, Bonnier Christopher et al. Use of segmented CT transmission map to avoid metal artifacts in PET images by a PET-CT device. BMC Nucl Med. 2005 Jun 14 [PMID: 15953395]

Yu Minjun, Qi Xiulan, Moreno Jose L et al. NF-kappaB signaling participates in both RANKL- and IL-4-induced macrophage fusion: receptor cross-talk leads to alterations in NF-kappaB pathways. J Immunol. 2011 Aug 15 [PMID: 21734075] (Mouse)


Details:
IKK-gamma NEMO Binding Domain (NBD) Inhibitor Peptide used for functional assay in vitro on K562 cells and it was found to selectively kill K562 ALDH+ cells (IC50: ALDH+, 40.52 um; ALDH-, 62.38 um) after 24 hours of treatment (Fig. S7).

Li Y. Mechanisms of vascular disease: divergent roles for suppressor of cytokine signaling 3 in angiotensin II-induced vascular dysfunction Thesis. 2014 (B/N, Mouse)

Salem K. Copper-zinc superoxide dismutase and glucose metabolism as redox targets for bortezomib resistance in multiple myeloma Thesis. 2014 (B/N, Human)


Details:
Fig 2A: Primary brain hippocampal cell [post synaptic density fractions( PSD)] cultures. Cultures were treated with 5 uM or 20 uM of the inhibitory peptide or 20 uM of the control peptide. Readout system: WB analysis of ATP induced-CLYD phosphorylation. The results showed that 20 uM of the inhibitory, but not control, peptide inhibited CYLD phosphorylation (using a CYLD phosphospecific antibody) and that total CYLD (using an antibody that recognized total CYLD) expression was unchanged. The results suggested that phosphorylation of CYLD requires the regulatory subunit of IKKgamma in the PSD hippocampal cell culture model system.


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