Product Datasheet

IKK gamma Inhibitor Peptide Set
NBP2-26504

Unit Size: 2 mg

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

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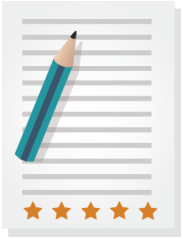
Updated 10/23/2024 v.20.1



NBP2-26504

IKK gamma Inhibitor Peptide Set

Product Information	
Unit Size	2 mg
Concentration	Lyoph
Storage	Store at -20C. Avoid freeze-thaw cycles.
Reconstitution Instructions	Please refer to the product PDF at novusbio.com for reconstitution instructions.

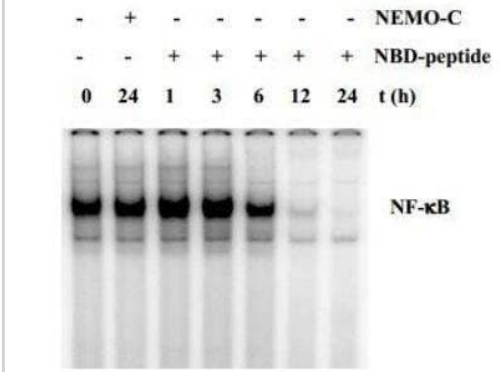
Product Description	
Gene ID	8517
Gene Symbol	IKBKG
Species	Human, Mouse, Rat
Specificity/Sensitivity	The IKK-gamma inhibitory peptide contains a protein transduction (PTD) sequence (DRQIKIWFQNRRMKWKK) derived from antennapedia which renders the peptide cell permeable (Derossi et al, The third helix of the antennapedia homeodomain translocates through biological membranes. J Biol Chem. 269:10444-10450 (1994)] .The control peptide consists of only the PTD sequence.
Immunogen	Functions as an IKK-Alpha/IKK-Beta decoy by binding to IKK-Gamma NBD, thereby preventing formation of the IKK complex.
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, Block/Neutralize
Recommended Dilutions	In vitro assay, In vivo assay, Functional (Inhibition) reported in scientific literature (PMID 24361600), Block/Neutralize reported in scientific literature (PMID 28166799)

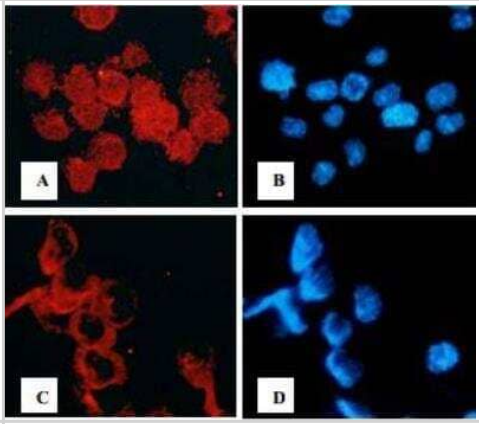
Application Notes	Inhibition of NF-kB activitiy in vivo and in vitro by interefering with IKK complex formation Researchers can study the effect of NBD inhibitor peptide using a variety of methods. Quantitative readout assays include NF-kB/p65 ActivELISA Kit, Phospho-IkBalpha ActivELISA Kit and EMSA. Immunocytochemistry can also be used as a readout assay for visualizing the subcellular localization of NF-kB; activated NF-kB localizes to the nucleus, whereas NF-kB in the cytoplasm is generally considered be 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 cells lines, including U266, have been found to have constitutively active NF-kB. The EMSA assay shows that NBD suppressed the constitutive activation of NF-kB in U266 cells. The mmunocytochemistry data provides supporting evidence that the nuclear translocation of NF-kB 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-kB is an important molecule for cell survival and proliferation, blockade of NF-kB 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.

Images

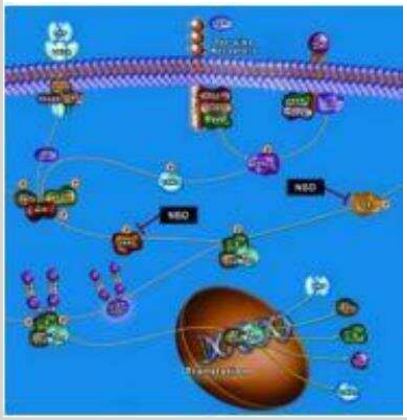
In vivo assay: IKK gamma Inhibitor Peptide Set [NBP2-26504] - NBD peptide blocks constitutive NF-kB as shown by EMSA. U266 cells were treated with 100 uM of control or NBD peptide for different time periods. Nuclear extracts were isolated and checked for NF-kB-DNA binding activity.



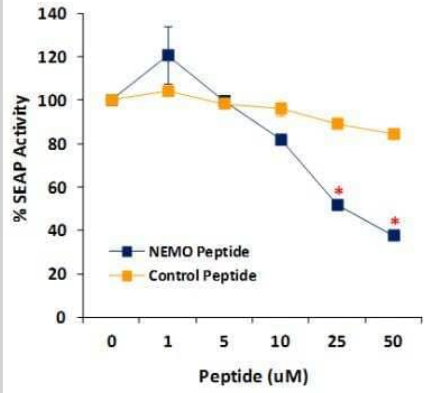
In vivo assay: IKK gamma Inhibitor Peptide Set [NBP2-26504] - NBD peptide blocks constitutive NF-kB activation in human multiple myeloma cells. U266 cells were treated with 100 uM of control (A & B) or NBD peptide (C & D) for 12 hr, cytospun, plated on glass slides, air dried for 1 hr at room temperature 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

Abuaita B, Schultz T, O'Riordan M. Mitochondria-Derived Vesicles Deliver Antimicrobial Reactive Oxygen Species to Control Phagosome-Localized Staphylococcus aureus. Cell Host & Microbe. 2018-10-01 [PMID: 30449314] (B/N, Mouse)

Alvarez-Carbonell D, Garcia-Mesa Y, Milne S et al. Toll-like receptor 3 activation selectively reverses HIV latency in microglial cells. Retrovirology. 2017-02-06 [PMID: 28166799] (B/N, Human)

Tanaka T, Imamura T, Yoneda M et al. Enhancement of active MMP release and invasive activity of lymph node metastatic tongue cancer cells by elevated signaling via the TNF-alpha-TNFR1-NF-kB pathway and a possible involvement of angiopoietin-like 4 in lung metastasis Int. J. Oncol. 2016-08-10 [PMID: 27511626]

Garvin LM, Chen Y, Damsker JM, Rose MC. A novel dissociative steroid VBP15 reduces MUC5AC gene expression in airway epithelial cells but lacks the GRE mediated transcriptional properties of dexamethasone. Pulm Pharmacol Ther. 2016-04-28 [PMID: 27133900] (Func, Human)

Li Y, Kinzenbaw DA, Modrick ML et al. Context-Dependent Effects of SOCS3 in Angiotensin II-Induced Vascular Dysfunction and Hypertension in Mice: Mechanisms and Role of Bone Marrow-Derived Cells. Am. J. Physiol. Heart Circ. Physiol. 2016-04-22 [PMID: 27106041] (Func, Mouse)

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. 2015-06-08 [PMID: 26060140] (Func, Mouse)

Majewski Pawel M, Thurston Robert D, Ramalingam Rajalakshmy et al. Cooperative role of NF-{kappa}B and poly (ADP-ribose) polymerase 1 (PARP-1) in the TNF-induced inhibition of PHEX expression in osteoblasts. J Biol Chem. 2010-11-05 [PMID: 20817730]

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. 2005-06-14 [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. 2011-08-15 [PMID: 21734075] (Mouse)

Zhang B, Shimada Y, Kuroyanagi J et al. In vivo selective imaging and inhibition of leukemia stem-like cells using the fluorescent carbocyanine derivative, DiOC5(3) Biomaterials 2015-06-01 [PMID: 25818410] (In-vitro, Func, Human)

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).

Salem K. Copper-zinc superoxide dismutase and glucose metabolism as redox targets for bortezomib resistance in multiple myeloma Thesis. 2014-01-01 [PMID: 25485927] (B/N, Human)

Thein S, Pham A, Bayer KU et al. IKK regulates the deubiquitinase CYLD at the postsynaptic density. Biochem. Biophys. Res. Commun. 2014-06-10 [PMID: 24928390] (B/N, Rat)

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.

More publications at http://www.novusbio.com/NBP2-26504

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H00008517-P01-10ug	Recombinant Human IKK gamma GST (N-Term) Protein
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NB100-56542	IKK gamma Antibody (72C627)
201-LB-005	IL-1 beta/IL-1F2 [Unconjugated]

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