

IKK-gamma Inhibitor Peptide Set

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| Catalog No: | NBP2-26504 |
| Content: | IKKg NEMO Binding Domain (NBD) Inhibitor Peptide: 2 x 1 mg (lyophilized) DRQIKIWFQNR ^{RMK} WKKTALDWSWLQTE (IKKg/NEMO binding sequence is underlined). Molecular weight: 3692 2 x 1 mg (lyophilized) |
| Control peptide: | DRQIKIWFQNR ^{RMK} WKK |
| Molecular weight: | 2361 |
| Species reactivity: | Human, Mouse, Rat |
| Storage: | The solid product is stable in the desiccator at room temperature or outside of the desiccator at -20C for 1 year. Once reconstituted, this peptide set should be stored at -80C in working volumes; with a shelf life of 6 months when stored properly. |
| Form: | White Solid |
| Application: | Inhibition of NF-kB activity in vivo and in vitro by interfering with IKK complex |
| Inhibitory mechanism: | Functions as an IKKa/IKKb decoy by binding to IKKg NBD, thereby preventing formation of the IKK complex. |
| Solubility: | Solubilize the peptides just prior to use, making 5 mM stock solutions. |

Background

IKKg binds to specific sequences on IKKa and IKKb, forming the IKK complex. The IKK complex phosphorylates downstream signaling molecules during NF-kB activation. The IKKg inhibitory peptide contains an IKKa and IKKb consensus binding sequence, called NEMO binding domain or NBD. When IKKa and IKKb are bound to the IKKg NBD peptide, they can not bind to IKKg. Thus the formation and function of the IKK complex is blocked. 1-2

IKKg NEMO Binding Domain (NBD) inhibitor peptide contains a protein transduction (PTD) sequence (DRQIKIWFQNR^{RMK}-WKK) derived from antennapedia which renders the peptide cell permeable.³ The control peptide consists of only the PTD sequence.

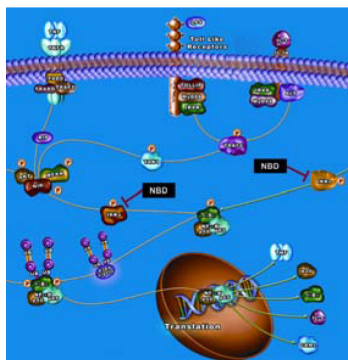


Figure 1. NBD perturbation of NF-kB signaling. The IKK complex consists of IKKa + IKKb + NEMO. NEMO (IKKy/NF-kB Essential MODulator) is a scaffold protein required for the kinase activity of IKKa + IKKb. NBD (NEMO Binding Domain) inhibits formation of the IKK complex (IKKa + IKKb + NEMO) by binding to NEMO and blocking IKK complex formation. In this regard, NBD is considered to be an "IKKa and IKKb decoy". Blocking IKK complex formation suppresses downstream events in the NF-kB signaling pathway that rely on the IKK complex including phosphorylation of Ikb α , and subsequent Ikb α ubiquitination, degradation and release of NF-kB. Hence, NF-kB is retained in the cytoplasm in its inactive state.

Research purposes only. Not for diagnostic or use in human. For use in animal, follow your Institution's Animal Handling Policy.

Preparation of 5 mM Stock Solutions

Note: Bring the peptide to room temperature and quick spin the tubes before opening the caps.

IKK γ NEMO Binding Domain (NBD) Inhibitor Peptide:

1 mg of DRQIKIWFQNRRMKWKKTALDWSWLQTE

A final volume of 54.2 μ l will make a 5 mM stock solution. Add 54.2 μ l of DMSO to the peptide. Carefully pipet to ensure all of the peptide is dissolved.

Control Peptide: 1 mg of DRQIKIWFQNRRMKWKK

A final volume of 84.8 μ l will make a 5mM stock solution. Add 84.8 μ l of DMSO to the peptide. Carefully pipet to ensure all of the peptide is dissolved.

Usage:

Researchers can study the effect of NBD inhibitor peptide using a variety of methods. Quantitative readout assays include NF- κ B/p65 ActivELISA™ Kit (NBP2-29661) and EMSA. Immunocytochemistry can also be used as a readout assay for visualizing the subcellular localization of NF- κ B; activated NF- κ B localizes to the nucleus, whereas NF- κ B 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- κ B (4). The EMSA assay shows that NBD suppressed the constitutive activation of NF- κ B in U266 cells. The immunocytochemistry data provides supporting evidence that the nuclear translocation of NF- κ B 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- κ B is an important molecule for cell survival and proliferation, blockade of NF- κ B 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.

In vivo assay:

1. Plate cells at 2×10^6 /ml in regular cell culture medium.
2. Add control or NBD peptide to a final concentration of 100 μ M for 12 hrs.
Example: 40 μ l of inhibitory peptide or control peptide stock solution, added to a final volume of 2 ml cell culture media, will give a final concentration of 100 μ M.
3. Prepare nuclear extracts and check for the presence of NF- κ B DNA-binding activity by EMSA (Figure 1).
Nuclear extracts can be prepared as described by Bharati A, et al., (Ref 2) or using NOVUS nuclear extraction kit (Cat. No. NBP2-29447).

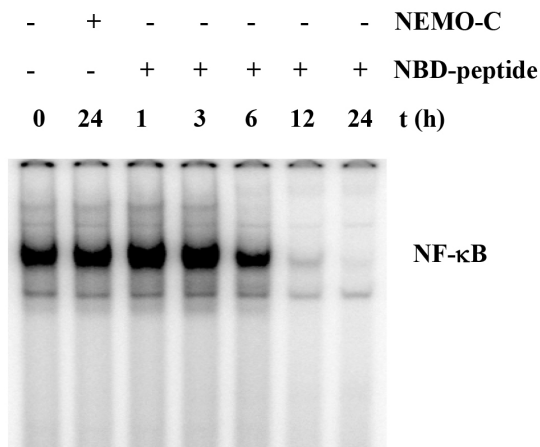


Figure 2: NBD peptide blocks constitutive NF- κ B as shown by EMSA. U266 cells were treated with 100 μ M of control or NBD peptide for different time periods. Nuclear extracts were isolated and checked for NF- κ B-DNA binding activity. (Courtesy of Dr. B.B. Aggarwal, MD Anderson Cancer Center, Houston, TX.)

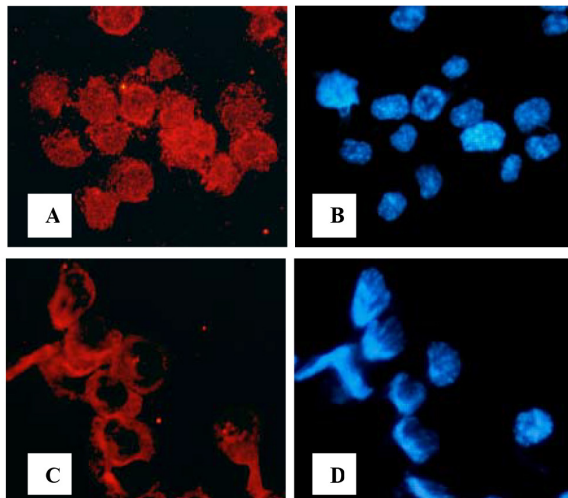


Figure 3: NBD peptide blocks constitutive NF- κ B activation in human multiple myeloma cells. U266 cells were treated with 100 μ M of control (A & B) or NBD peptide (C & D) for 12 hr, cytospun, plated on glass slides, air dried for 1 hr at room temperature and fixed with cold acetone. Slides were blocked 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.

(Courtesy of Dr. B.B. Aggarwal, MD Anderson Cancer Center, Houston, TX.)

References:

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