NFkB p65 [p Ser276] Inhibitor Peptide Set

Catalog No:	**NBP2-26505**
Content:	**NFkB p65 [Ser276] Inhibitor peptide: 2 x 1 mg (lyophilized)** DRQIKIWFQNRRMKWKKQLRRPSDRELSE (p65 sequence is underlined). Molecular weight: 3830 **Control peptide: 2 x 1 mg (lyophilized)** DRQIKIWFQNRRMKWKK Molecular weight: 2361.
Storage:	The solid product is stable in the dessicator at room temperature for 1 year. However, we recommend storing dessicated at -20 degree C.
Species Reactivity:	Cow, Dog, Human, Most Mammals, Mouse, Rat
Form:	White Solid

Inhibitor Mechanism

Functions as a p65 decoy through phosphorylation of the Ser276 site on the peptide Application: Inhibition of DNA binding activity of NFkB.

Background

NF-kB has been shown to regulate the expression of a number of genes whose products are involved in inflammation, viral replication, carcinogenesis, antiapoptosis, invasion, and metastasis. Specific adhesion molecules, chemokines, inflammatory cytokines, and cell cycle regulatory genes are affected. Thus, agents that can suppress NF-κB activation have the potential to be treatments for inflammatory diseases and cancer.

The Ser276 site of p65 is phosphorylated during NFkB activation, allowing p65 nuclear translocation. This p65 inhibitory peptide contains a Ser276 site that is phosphorylated during NFkB activation, thereby blocking p65 Ser276 phosphorylation (Bharti et al, 2003; Takada et al, 2004). The NFkB p65 (Ser276), also known as PTD-p65-P1, inhibitory peptide also contains a protein transduction (PTD) sequence (DRQIKIWFQNRRMKWKK) derived from antennapedia which renders the peptide cell permeable (Derossi et al, 1994). The control peptide consists of only the PTD sequence. The PTD-p65-P1 peptide can inhibit binding of recombinant p65 protein to the DNA in a dose dependent manner and maximum inhibition occurs at 50 μM (Figure 1). It can also inhibit TNF- induced NF-κB activation in vivo. PTD-p65-P1 suppresses TNF-induced NF-κB activation by 25% at 100 μM and completely at 150 μM (figure 2).

Broad: Peptide sequence is 100% conserved across multiple species. Reactivity includes human, mouse, rat, dog, and cow. Solubility Solubilize the peptides prior to use by making 5 mM PBS* stock solutions (please see Preparation of 5 mM Stock Solutions). The stock solutions are stable at -20 degree C for 6-8 months. Avoid repeated freeze/thaw cycles. For multiple uses, we suggest aliquoting the stock solution prior to freezing.

Preparation

Preparation of 5 mM Stock Solutions:

PBS* is added directly to the vials to prepare the stock solutions.
Note: Bring the solution to room temperature and quick spin the tubes before opening the caps.

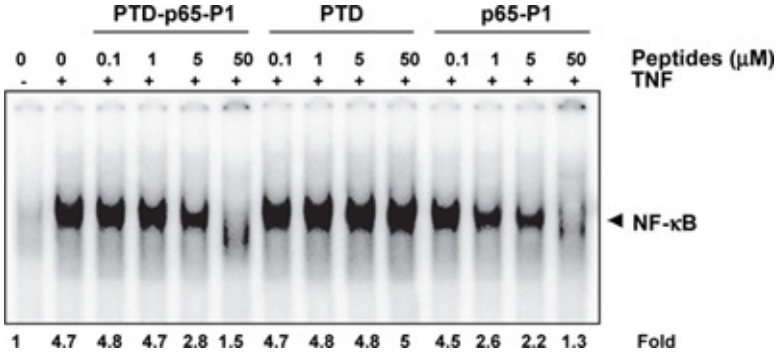
NF-kB p65 (Ser276) Inhibitor Peptide: 1 mg of DRQIKIWFQNRRMKWKKQLRRPSDRELSE)
Add 52.2 ul of PBS* to the vial to make a 5 mM stock solution.
Mix by vortexing.
Aliquot and store at -20 degree C or -80 degree C.
Avoid repeated freeze thawing.

Control Peptide: 1 mg of DRQIKIWFQNRRMKWKK
Add 84.8 ul PBS* to the vial.
Mix by vortexing.
Aliquot and store at 20 degree C or -80 degree C.
Avoid repeated freeze thawing.

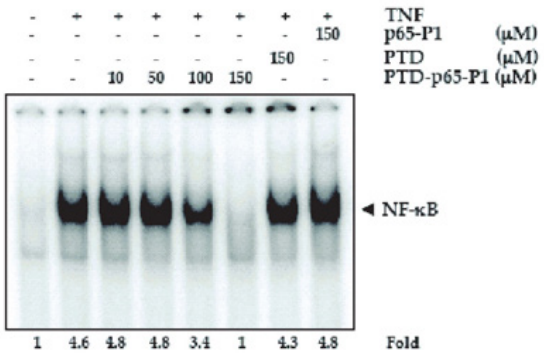
***Recipe for 1X PBS:**
1. Dissolve the following in 800ml distilled H2O:
   8g of NaCl, 0.2g of KCl
   1.44g of Na2HPO4
   0.24g of KH2PO4
2. Adjust pH to 7.5 with HCl.
3. Adjust volume to 1L with additional distilled H2O.
4. Sterilize by autoclaving

Usage

Researchers can study the effect of p65 inhibitor peptide using a variety of methods. This is a general protocol and may need to be optimized. Preincubate cells with appropriate amounts of inhibitory or control peptides for 1 hr and then treat with TNF or other NFkB activating agents. Prepare nuclear extracts and check for the presence of NFkB DNA-binding activity by EMSA (Figure 1). Nuclear extracts can be prepared as described by Bharati A, et al. and Takada et al or using Novus nuclear extraction kit (Cat.# NBP2-29447). Please refer to Takada et al, 2004 for further details on the use of this inhibitory peptide.



Effect of various p65-inhibitor peptides on binding of purified recombinant p65 protein to the DNA in vitro. Recombinant p65 protein (100 ng/sample) was incubated for 30 minutes with 50μM of various peptides in 0.025 ml and then assayed for DNA binding activity by EMSA (Please refer to Takada et al, 2004 for further details).



The PTD-p65-P1 peptide inhibits TNF-induced NFkB activation: KBM-5 cells were incubated with various concentrations of peptides for 1 hr and treated with 0.1nM TNF for 30 min. Nuclear extracts were prepared and NF-kB activation was analyzed by EMSA (Please refer to Takada et al, 2004 for further details).

Product Citations

1. Identification of a p65 peptide that selectively inhibits NF-κB activation induced by various inflammatory stimuli and its role in down-regulation of NF-κB-mediated gene expression and upregulation of apoptosis. Takada Y, Singh S, and Aggarwal, BB. *J Biol Chem* 279: 15096-15104 (2004).
2. TNFα potentiates glutamate neurotoxicity by inhibiting glutamate uptake in organotypic brain slice cultures: neuroprotection by NF-κB inhibition. Jian Y. Zou and Fulton T. Crews. *Brain Research,* 1034 (1-2): 11-24 (2005).
3. Curcumin (diferuloylmethane) inhibits constitutive NF-κB activation, induces G1/S arrest, suppresses proliferation, and induces apoptosis in mantle cell lymphoma. Shishir Shishodia, Hesham M. Amin, Raymond Lai and Bharat B. Aggarwal. *Biochemical Pharmacology, Volume 70* (5): 700-713 (2005). SummaryPlus | Full Text + Links | PDF (704 K)
4. The role of CaMKII in BDNF-mediated neuroprotection of retinal ganglion cells (RGC-5). Wei Fan, Neeraj Agarwal and Nigel G.F. Cooper. Brain Research, In Press, Corrected Proof, Available online 6 December 2005.
5. Guggulsterone Inhibits Osteoclastogenesis Induced by Receptor Activator of Nuclear Factor-κB Ligand and by Tumor Cells by Suppressing Nuclear Factor-κB Activation. Haruyo Ichikawa and Bharat B. Aggarwal. Clin. *Cancer Res.*, 12: 662-668 (2006).
6. Toll-like receptor 2 is required for inflammatory response to Francisella tularensis LVS. Katz J, P Zhang, M Martin, S Vogel, S Michalak. Infection and Immunity 74: 2809-2816 (2006). NBP2-26505: (mouse dentritic cells), Figs. 5, 6.
7. TRIP, a novel molecular partner of the MAGI-1 scaffolding molecule, promotes invasiveness. Chastre E, M Abdessamad, A Kruglov, E Bruyneel, BM Bracke, Y Di Giola, M Beckerle, F van Roy, L Kotelevets. *FASEB doi*: fj.08-106344 (2008). HeLa and HEK293 cell lysates were transiently transfected with Flag labeled MAG-1b.
8. The NF-kB inhibitor curcumin blocks sepsis-induced muscle proteolysis. Poylin V, MU Fareed, P 0\'Neal, N Alamdari, N Reilly, M Menconi, and P-O Hasselgren. *Mediators of Inflammation doi*:10.1155/2008/317851 (2008). incubated muscles from septic rats, muscles were incubated in the presence of 100 um inhibitory peptide or 100 um control peptide. Treatment of the muscles with the inhibitory peptide for 2 hr resulted in an ~30% reduction of protein breakdown compared to incubation with the control peptide as measured by a tyrosine release assay.
9. Anisotropic regulation of Ankrd2 gene expression in skeletal muscle by mechanical stretch. Mohamed J, M Lopez, G Cox, A Boriek. F*ASEB J doi*: 10.1096/fj.10-158386 (2010). Novus products cited:
    1. NF-kB p50 (NLS) Inhibitory Peptide Set (NBP2-29323): Fig 4AB
    2. NF-kB p65 (Ser276) Inhibitory Peptide Set (NBP2-26505): Fig 4AB

    NOTE: Wild type mouse diaphragms were incubated with p50 and p65 inhibitory peptides, then nuclear and cytoplasmic lysates were subject to western blot with normal and phospho-specific p50 or p65 antibodies. The assays showed that the p50 (NBP2-29323) and p65 (NBP2-26505) peptides inhibited p50 and p65 phosphorylation, respectively.
10. Interaction with vascular endothelium enhances survival in primary chronic lymphocytic leukemia cells via NF-kB activation and De novo gene transcription. Buggins A, C Pepper, P Patten, S Hewamana, S Gohil, J Moorhead, N Folarin, D Yallop, N Thomas, G Mufti, C Fegan, S Devereux. *Cancer Res* 19:7523-7533 (2010). **Novus product cited: p65 inhibitory peptide (NBP2-26505). CLL (chronic lymphocytic leukemia) cells were incubated with p65 inhibitory peptide and cultured in the presence or absence of endothelial cells, Fig 5.**
11. Convergence of the mammalian target of rapamycin complex 1 and glycogen synthase kinase 3-beta signaling pathways regulates the innate inflammatory response. Wang H, J Brown, Z Gu, C Garcia, R Liang, P Alard, E Beurel, R Jope, T Greenway, M Martin. *J Immunol* 186:5217-5226 (2011). **WB: Human PBMC's were pretreated with the NF-kB p65 inhibitory peptide then treated with Rapamycin in the presence or absence of LPS, Fig 5B.**
12. Curcuumin (Diferuloylmethane) Inhibits Constitutive and IL-6-Inducible STAT3 Phosphorylation in Human Myeloma Cells. Alok C. Bharti, Nicholas Donato, and Bharat B. Aggarwal. *J.Immunol* 171:3863-3871 (2003).
13. The Third Helix of the Antennapedia Homeodomain Translocates through Biological Membranes. Derossi D, AH Joliot, G Chassings, A Prochiantz. *J Biol Chem.* 269:10444- 10450 (1994).

14. c-Met and NF-kappaB-dependent overexpression of Wnt7a and -7b and Pax2 promotes cystogenesis in polycystic kidney disease. Qin S, M Taglienti, L Cai, J Zhou, JA Kreidberg. *J Am Soc Nephrol* 23: 1-10 (2012). **Novus products cited**:

    1. **IKK-gamma NEMO Binding Domain Inhibitory Peptide (NBP2-26504): Fig 6E (immortalized mouse kidney epithelial cell line) and Fig 6F (mouse kidney explants). The cell line and explants were treated with NBP2-26504. Readout assay: RT-qPCR for Wnt7a and 7b, and Pax2. Fig 6G & H (mouse kidney explants). Readout assay: IHC (P). Cysts were quantified on hematoxylin/esoin tissue sections.**

    2. **NF-κB p65 Inhibitory Peptide (NBP2-26505). Similar experiments as those described for NBP2-26505, similar results obtained. Data described but not shown.**