NFkB p65 [p Ser276] Inhibitor Peptide Set

Catalog No:	**NBP2-26505**
Content:	**Inhibitor peptide: 2 x 1 mg (lyophilized)** DRQIKIWFQNRRMKWKKNGLLSGDEDFSS (p65 sequence is underlined). Molecular weight: 3697.21 **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°C.
Species Reactivity:	Broad: Peptide sequence is 100% conserved across multiple species. Reactivity includes human, mouse, rat, dog, and cow.
Form:	White Solid
Application:	Inhibition of NFkB activitiy.
Inhibitory mechanism:	Functions as a p65 decoy through phosphorylation of the Ser529/536 sites on the peptide.
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°C for 6-8 months. Avoid repeated freeze/thaw cycles. For multiple uses, we suggest aliquoting the stock solution prior to freezing.

Background

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

The Ser529/536 sites of p65 are phosphorylated during NFkB activation, allowing p65 nuclear translocation. This p65 inhibitory peptide contains Ser529/536 sites that are phosphorylated during NFkB activation, thereby blocking p65 Ser529/536 phosphorylation. The NFkB p65(Ser529/536) Inhibitor Peptide can inhibit binding of recombinant p65 protein to the DNA in a dose dependent manner and maximum inhibition occurs at 50 uM (Figure 1). It can also inhibit TNF-induced NFkB activation in cells (Figure 2).

The NFkB p65(Ser529/536) inhibitory peptide also contains a protein transduction (PTD) sequence (DRQIKIWFQNRRMK-WKK) derived from antennapedia which renders the peptide cell permeable.[2] The control peptide consists of only the PTD sequence.

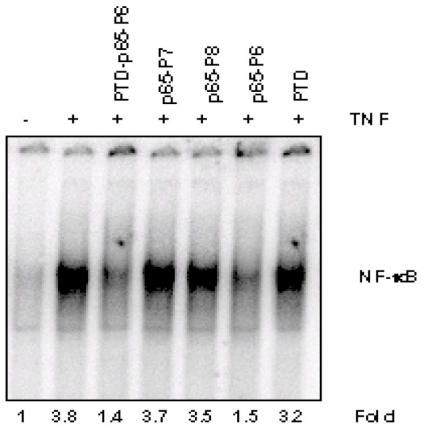

Figure 1: Effect of various p65-inhibitor peptides on binding of purified recombinant p65 protein to the DNA in vitro. Recombinant p65 protein (100 ng/sample) was incubated for 30 minutes with 50 uM of various peptides in 0.025 ml and then assayed for DNA binding activity by EMSA (Please refer to Takada et al, 2004 (3) for further details).

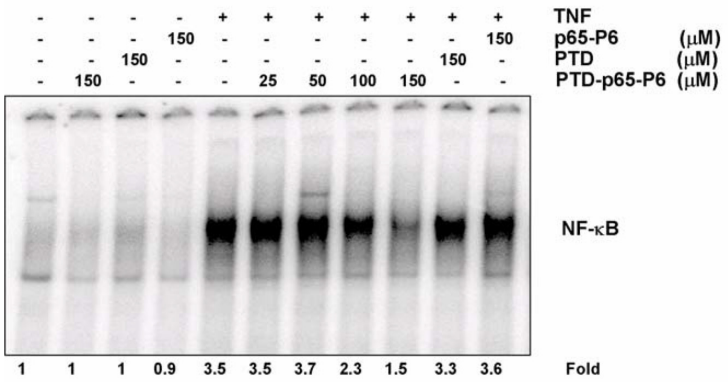

Figure 2: The NFkB p65(Ser529/536) Inhibitor Peptide inhibits TNF-induced NFkB activation: KBM-5 cells were incubated with various concentrations of peptides for 1 hr and treated with 0.1nM TNF for 30 min. Nuclear extracts were prepared and NFkB activation was analyzed by EMSA (Please refer to Takada et al, 2004 for further details). PTD-p65-P6 suppresses TNF-induced NFkB activation by 25% at 100 uM and completely at 150 uM, where as the control peptide, PTD or p65-P6 without PTD did not have any effect.

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.*

NFkB p65(Ser529/536) Inhibitor Peptide:
1mg of DRQIKIWFQNRRMKWKKNGLLSGDEDFSS

Add 54.1 ul PBS* to the vial, to make a 5 mM stock solution. Mix by vortexing. Aliquot and store at -20°C or -80°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 20oC or -80oC. 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. Following is a general protocol for KBM-5 cells. It may need to be optimized for different cell types.

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 DNAbinding activity by EMSA (Figure 1). Nuclear extracts can be prepared as described by Bharati A, et al, 2003 (3) and Takada et al, 2004 (4) or using Novus nuclear extraction kit (Cat. No. NBP2-29447). Please refer to Takada et al, 2004 for further details on the use of this inhibitory peptide.

Reference:

1. SR Schwarze, KA Hruska, SF Dowdy. Protein transduction: unrestricted delivery into all cells? *Trends Cell Biology*, 10 (7): 290-295 (2000).
2. Derossi D, AH Joliot, G Chassaings, A Prochiantz. The Third Helix of the Antennapedia Homeodomain Translocates through Biological Membranes. *J Biol Chem.* 269:10444-10450 (1994).
3. Bharti AC., N Donato, and B. Bharat Aggarwal Curcumin (Diferuloylmethane) Inhibits Constitutive and IL-6-Inducible STAT3 Phosphorylation in Human Multiple Myeloma Cells.. *J. Immunol.,* 171: 3863-3871 (2003).
4. Takada Y, S Singh, and BB Aggarwal,. Identification of a p65 peptide that selectively inhibits NFkB activation induced by various inflammatory stimuli and its role in down-regulation of NFkBmediated gene expression and upregulation of apoptosis. *J Biol Chem* 279: 15096-15104 (2004).