

## NFkB p105/p50 Inhibitor Peptide Set

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<b>Catalog No:</b>	<b>NBP2-29323</b>
<b>Content:</b>	<b>NFkB p50 (NLS) Inhibitor peptide: 2 x 1 mg (lyophilized)</b> DRQIKIWFQNRRMKWKK <u>VQRKRQKLM</u> (p50 NLS sequence is underlined). Molecular weight: 3529  <b>Control peptide: 2 x 1 mg (lyophilized)</b> DRQIKIWFQNRRMKWKK Molecular weight: 2361
<b>Storage:</b>	The solid product is stable in the dessicator at room temperature for 1 year. However, we recommend storing dessicated at -20°C.
<b>Species Reactivity:</b>	Broad: Peptide sequence is 100% conserved across multiple species. Reactivity includes human, mouse, rat, dog, cow, chicken, and xenopus
<b>Form:</b>	White Solid
<b>Application:</b>	Inhibition of NF-kB p50(NLS) activity.
<b>Inhibitory mechanism:</b>	Functions as a p50 decoy by blocking the intracellular recognition mechanism of p50 NLS.
<b>Solubility:</b>	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

The NLS sequence is required for nuclear translocation of NFkB p50. In stimulated cells, NFkB is in the cytoplasm in an inactive form and the NLS is masked. The degradation of Ikb $\alpha$  during NFkB signaling unmask the NLS of NFkB subunits and NFkB is translocated to the nucleus. The p50 inhibitory peptide contains the p50 NLS sequence (VQRKRQKLM) and blocks the intracellular recognition mechanism for the NLS present on p50. Consequently p50 nuclear translocation is blocked.<sup>1</sup>

The p50 NLS inhibitory peptide also contains a protein transduction (PTD) sequence (DRQIKIWFQNRRMKWKK) derived from antennapedia which renders the peptide cell permeable.<sup>2</sup> The control peptide consists of only the PTD sequence.

*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

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.*

### **p50 (NLS) Inhibitor Peptide: 1 mg of DRQIKIWFQNRRMKWKKVQRKRQKLM**

Add 57 ul of 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 20°C or -80°C. Avoid repeated freeze thawing.

### **\*Recipe for 1X PBS:**

1. Dissolve the following in 800ml distilled H<sub>2</sub>O.
  1. 8g of NaCl
  2. 0.2g of KCl
  3. 1.44g of Na<sub>2</sub>HPO<sub>4</sub>
  4. 0.24g of KH<sub>2</sub>PO<sub>4</sub>
2. Adjust pH to 7.5 with HCl.
3. Adjust volume to 1L with additional distilled H<sub>2</sub>O.
4. Sterilize by autoclaving

### **Usage:**

The inhibitor peptide is designed to block p50 nuclear translocation. Optimal peptide concentrations and incubation times vary between model systems and should be determined empirically by users. A 100 uM final concentration may be a useful starting point. Please refer to Lin et al (1995) for additional information about how the inhibitor peptide has been used to block p50 nuclear translocation.

### **Reference:**

1. Lin Y-Z., SY Yao, RA Veach, TR Torgerson, J Hawiger. Inhibition of nuclear translocation of transcription factor NFkB by a synthetic peptide containing a cell membrane-permeable motif and nuclear localization sequence. *J Biol. Chem.* 270:14255-14258 (1995).
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).