

## ERK Inhibitory Peptide Set

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<b>Catalog No.:</b>	<b>IMG-2008</b>
<b>Content:</b>	<b>ERK Inhibitor peptide: 2 x 1 mg (lyophilized)</b> DRQIKIWFQNR <b>RMKWKK</b> <u>GMPKKKPTPIQLN</u> (ERK inhibitor sequence is underlined). Molecular weight: 3795.  <b>Control peptide: 2 x 1 mg (lyophilized)</b> DRQIKIWFQNR <b>RMKWKK</b> Molecular weight: 2361
<b>Species Reactivity:</b>	Broad; the peptide sequence is 100% conserved among multiple species. Reactivity includes human, mouse, rat, hamster, rabbit, and xenopus.
<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>Form:</b>	White Solid
<b>Application:</b>	Inhibition of Erk activation.
<b>Inhibitory Mechanism:</b>	Functions as a MEK decoy by binding to ERK.
<b>Solubility:</b>	Solubilize the peptides prior to use by making 5 mM PBS* stock solutions (please see <b>Preparation of 5 mM Stock Solutions</b> ). 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**

ERK (extracellular signal-regulated kinase) is a member of the Mitogen-activated protein kinases (MAPK) family of protein kinases that are essential for cellular proliferation and differentiation. The activation of MAPKs requires a cascade mechanism whereby MAPK is phosphorylated by an upstream kinase MAPKK (MEK) which is then, in turn phosphorylated by a third kinase MAPKKK (MEKK). This inhibitory peptide contains the amino-terminal 13 amino acids (GMPKKKPTPIQLN) of MEK1 and binds to ERK.<sup>1</sup> This blocks ERK activation by MEK as ERK is unable to interact with MEK.

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

## **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.*

### **ERK Inhibitor Peptide: 1 mg of DRQIKIWFQNRRMKWKKGMPKKKPTPIQLN**

Add 53 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.
  - 8g of NaCl
  - 0.2g of KCl
  - 1.44g of Na<sub>2</sub>HPO<sub>4</sub>
  - 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 to block ERK activation by MEK. 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 Kalemien et al (2002) for additional information about how the inhibitor peptide has been used to block ERK activation by MEK.

### **Reference:**

1. Kelemen BR, K Hsiao, SA Goueli. Selective *in vivo* inhibition of mitogen-activated protein kinase activation using cell-permeable peptides. *277:8741-8748* (2002).
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).