



TIRAP (TLR2 and TLR4) Inhibitor Peptide Set

Catalog No:	NBP2-29331
Content:	TIRAP Inhibitor peptide: 2 x 1 mg (lyophilized) DRQIKIWFQNRRMKWKK <u>LQLRDAAPGGAIVS</u> (TIRAP sequence is underlined). Molecular weight: 3701.4. 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:	Human, Mouse (the inhibitor peptide sequence is from mouse and also reacts with human; there is only one amino acid difference between the mouse and human sequence)
Form:	White Solid
Application:	Inhibition of TIRAP binding to TLR2 or TLR4
Inhibitory mechanism:	Functions as a TIRAP decoy by binding to TIR interacting domains on specific TLR receptors.
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

TIRAP/Mal is an adapter protein in the signaling pathways activated by TLR2 and TLR4, and appears to be essential for MyD88-dependent TLR2 and TLR4 signaling pathways. TIRAP is recruited to activated TL2 and TLR4 through interaction with TIR domain of the receptor. This peptide contains a sequence from mouse TIRAP that blocks the function of TIRAP, likely through binding to the receptor and blocking TIR-TIR domain interaction between TIRAP and the receptor.¹

The TIRAP inhibitory peptide contains a protein transduction (PTD) sequence (DRQIKIWFQNRRMKWKK) derived from antenapedia which renders the peptide cell permeable.² 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.

TIRAP Inhibitor Peptide: 1 mg of DRQIKIWFQNRRMKWKKLQLRDAAPGGAIVS

Add 54 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 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:

Inhibitory peptide at 100 uM concentration may be a starting point. However, useful concentration of peptide may vary depending on experimental condition. Incubate cells for 24 hr with peptides before stimulating with ligands.

Reference:

1. Schilling D, K Thomas, K Nixdorff, SN Vogel, MJ Fenton. Toll-like receptor 4 and Toll-IL-1 receptor domain-containing adapter protein (TIRAP/myeloid differentiation protein 88 adapter-like (Mal) contribute to maximal IL-6 expression in macrophages. *J Immunol.* 169:5874-5880 (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).

Product Citation:

Ganglioside GD1a is an essential coreceptor for Toll-like receptor 2 signaling in response to the B subunit of type IIb enterotoxin. Liang S, M Wang, RI Tapping, V Stepensky, HF Nawar, M Triantafilou, K Triantafilou, TD Connell, G Hajishengallis. *J Biol Chem* 282:7532-7542. (2006) (Human monocytes, Fig. 6A: block ability of LT-IIb-B5 to induce IL-6 production); Specificity of the inhibitory TIRAP blocking peptide confirmed by finding that peptide did not influence IL-6 induction by a TLR9 agonist CpG ODN (Fig. 6A).

β 2-integrin induced p38MAPK activation is a key mediator in the CD14/TLR4/MD2-dependent uptake of LPS by hepatocytes. Scott M, T Biliar. *J Biol Chem* 283:29433-29446 (2008).

Novus Biologicals products cited:

1. MyD88 (NBP2-29328): MyD88 peptide inhibition (mouse WT hepatocytes), Figs. 4A,D.
2. TIRAP (NBP2-29331): TIRAP peptide inhibition (mouse WT hepatocytes), Figs. 4B,D; 5B; 6E.