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TIRAP Inhibitory Peptide Set

Catalog No.:	IMG-2006-5
Content:	TIRAP Inhibitor peptide: 5 x 1 mg (lyophilized) DRQIKIWFQNRRMKWKK <u>LQLRDAAPGGAIVS</u> (TIRAP sequence is underlined). Molecular weight: 3701.4. Control peptide: 5 x 1 mg (lyophilized) DRQIKIWFQNRRMKWKK Molecular weight: 2361.
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)
Storage:	The solid product is stable in the dessicator at room temperature for 1 year. However, we recommend storing dessicated at -20°C.
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 TLR2 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 antennapedia which renders the peptide cell permeable.² 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.*

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 agonsit CpG ODN (Fig. 6A).

β2-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).

Imgenex products cited:

1. MyD88 (IMG-2005): MyD88 peptide inhibition (mouse WT hepatocytes), Figs. 4A,D.
2. TIRAP (IMG-2006): TIRAP peptide inhibition (mouse WT hepatocytes), Figs. 4B,D; 5B; 6E.