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TLR2 and TLR4 Inhibitor Peptide: COBRA

Catalog No.: NBP2-26245

Contents: TLR2 / TLR4 Inhibitor Peptide: 1 mg (lyophilized); sequence: DRQIKIWFQNRRMKWKKPGFLRDPWCKYQML (Inhibitor sequence is underlined); Molecular weight: 4097.92 Da
Control Peptide: 1 mg (lyophilized); sequence: DRQIKIWFQNRRMKWKK; Molecular weight: 2361 Da

Storage: The TLR2 /4 lyophilized inhibitor and control are stable in the desiccator at -20 oC for 1 year. DMSO-reconstituted solution is stable for up to two months at -20oC.

Background:

Toll/interleukin-1 receptor (TIR) domain-containing adapter protein/MyD88 adapter-like (TIRAP/Mal) is an adapter protein that facilitates recruitment of MyD88 to TLR2 and TLR4 signaling complexes. The TLR2 and TLR4 inhibitor is derived from the TIRAP/Mal adapter and blocks both TLR2 and TLR4 signaling by interfering with signaling complex assembly as a decoy in human and mouse (Figures 1 through 3; Also refer to the reference 1). This peptide is highly specific for TLR2 and TLR1 complex but not for TLR2 and TLR6 complex1 (Figure 1). The inhibitor contains a protein transduction (PTD) sequence (DRQIKIWFQNRRMKWKK) derived from antennapedia which facilitates internalization of the peptides2 while control consists only of the PTD.

Solubility:

Solubilize the peptides prior to use by preparing 2.5 mM stock solution in sterile water. The stock solutions are stable at -20oC or -80oC for at least two months; avoid repeated freeze/ thaw cycles and store in a non frost free freezer.

Preparation:

Note: Bring the peptides to room temperature and quick spin the tubes before opening the caps.
TLR2 / TLR4 Inhibitor Peptide: A final volume of 100 ul will make a 2.5 mM stock solution. Add 100 ul sterile water to the tube of peptide. Carefully pipet to ensure all of the peptide is dissolved and briefly spin the tube before opening.
Control Peptide: A final volume of 170 ul will make a 2.5 mM stock solution. Add 170 ul sterile water to the tube of peptide. Carefully pipet to ensure all of the peptide is dissolved and briefly spin the tube before opening.
The stock solutions may be diluted further to make working solutions. Dilute according to the needs for your assay. For example, dilute 2.5 mM stock solutions 1:10 in sterile 1X PBS or cell culture media to make 250 uM working solutions. Working solution should be made fresh daily and not be stored.

Usage:

The inhibitor is used in assays to inhibit TLR2 and TLR4 signaling. We recommend an initial titration of the inhibitor from 0-50 uM for in vitro assays along with control of which concentrations should be mirror inhibitor concentrations. Inhibitor and control should be preincubated with cells prior to ligand activation to allow sufficient time for the peptides to enter from the media into the cell. We typically preincubate with inhibitor and control for 1 h prior to TLR2 or TLR4 activation (Figures 1 and 2); however, optimal preincubation times may vary between model systems.
The TLR2/NF-kB/SEAPorterTM cell line (NBP2-26274) and TLR4/MD2/CD14/NF-kB/SEAPorterTM cell line (NBP2-26503) are a useful positive control model system for studying inhibition of TLR2 and TLR4 activation, respectively (Figures 1 through 3).

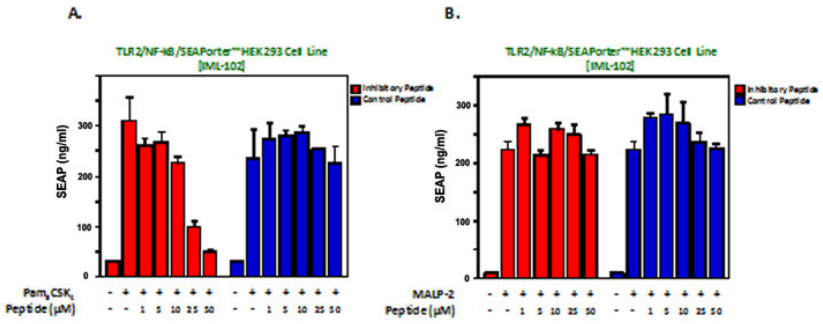

Figure 1. Evaluation of TLR2 inhibitor activity on signaling. TLR2 /NF-kB/ SEAPorter HEK 293 (NBP2-26274) cells were plated in 96-well plates at 7.5 x 10^4 cells/well for 16 h. Cells were preincubated with different concentrations of inhibitor and control (0, 1, 5, 10, 25 and 50 uM) for 1 h. Cells were then stimulated with 0.5 ng/ml Pam3CSK4 (NBP2-25297) [A] or 0.1 ng/ml MALP-2 (NBP2-26219) [B] for 24 h. Secreted alkaline phosphatase (SEAP) was analyzed using SEAPorter Assay Kit (NBP2-25285).
Data Summary: TLR2 acts through formation of heterodimer complexes with TLR1 or TLR6. HEK 293 endogenously expresses TLR1 and TLR6, so that the TLR2 reporter cell line (NBP2-26274) can response to both Pam3CSK4 (TLR2/TLR1 specific ligand) and MALP-2 (TLR2/TLR6 specific ligand). The TLR2 / TLR4 inhibitor specifically inhibits TLR2/TLR1 receptor complex activity in a dose-response manner but exhibited no or little effect on TLR2/TLR6 receptor complex activity (Refer to reference 1).

Research purposes only. Not for diagnostic use.

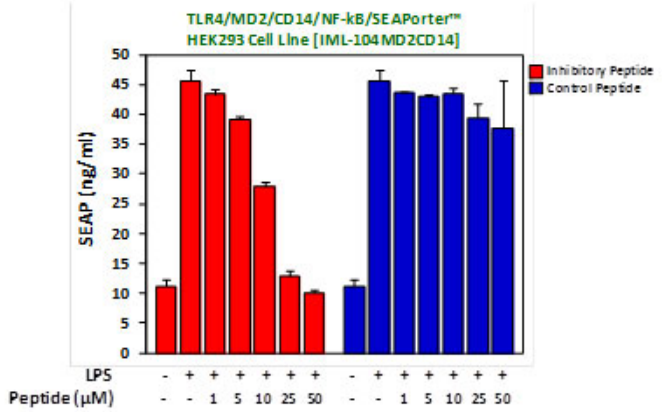

Figure 2. Evaluation of inhibitor activity on TLR4 signaling. TLR4/MD2/CD14/NF-kB/SEAPorter HEK 293 (NBP2-26503) cells were plated in 96-well plates at 7.5 x 10^4 cells/well for 16 h. Cells were preincubated with different concentrations of inhibitor and control (0, 1, 5, 10, 25 and 50 μM) for 1 h. Cells were then stimulated with 100 ng/ml LPS (NBP2-31066) for 24 h. Secreted alkaline phosphatase (SEAP) was analyzed using SEAPorterTM Assay Kit (NBP2-25285).
Data Summary: The TLR4 reporter cell line (NBP2-26503) responds to LPS. The TLR2 / TLR4 inhibitor specifically inhibits TLR4 activation upon LPS stimulation in a dose-response manner.

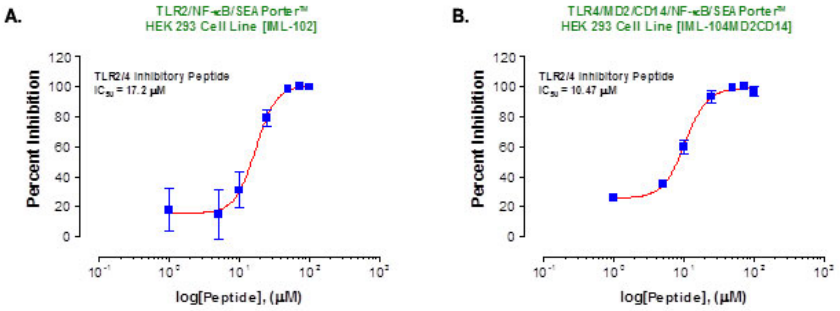

Figure 3. IC50 evaluation of the TLR2 /4 inhibitor. TLR2 /NF-kB/SEAPorter HEK 293 (NBP2-26274) cells and TLR4/MD2/CD14/NF-kB/SEAPorter HEK 293 (NBP2-26503) cells were plated in 96-well plates at 7.5 x 10^4 cells/well for 16 h. Cells were preincubated with various concentrations of inhibitor for 1 h. Cells were then stimulated with 0.5 ng/ml Pam3CSK4 (NBP2-25297) [A] or 100 ng/ml LPS (NBP2-31066) [B] for 24 h. Secreted alkaline phosphatase (SEAP) was analyzed using SEAPorter Assay Kit (NBP2-25285). Dose-responsive percent inhibition of each sample well was calculated to yield the peptide IC50 values.
Data Summary: TLR2 /4 Inhibitor suppressed the Pam3CSK4-induced TLR2/TLR1 activity [A] and the LPS-induced TLR4 activity [B] in a dose-response manner, of which IC50 values were measured as 17.2 uM and 10.47 uM, respectively.

Reference:

1. Couture, L. A. et al. (2012). Targeting Toll-like receptor (TLR) signaling by Toll/Interleukin-1 receptor (TIR) domain-containing adapter protein/MyD88 adapter-like (Mal)-derived decoy peptides. J. Biol. Chem. 287, 24641-24648.

2. Derossi, D. et al. (1994). The third helix of the Antennapedia homeodomain translocates through biological membranes. J. Biol. Chem. 269, 10444-10450.