

Pepinh-MYD

MyD88 Inhibitory Peptide

Catalog # tlr1-pimyd

For research use only

Version # 09J28-MT

PRODUCT INFORMATION

Content:

- 2 mg of lyophilized Pepinh-MYD
- 2 mg of lyophilized Pepinh-Control
- 2 x 1.5 ml endotoxin-free water

Storage and stability:

- Pepinh-MYD and Pepinh-Control are provided lyophilized and shipped at room temperature. Store at -20°C. Lyophilized product is stable 1 year at -20°C.
- Upon resuspension, Pepinh-MYD and Pepinh-Control should be aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles. Resuspended product is stable for 6-8 months at -20°C when properly stored.

DESCRIPTION

Pepinh-MYD is a 26 aa peptide that blocks MyD88 signaling by inhibiting its homodimerization through binding. Pepinh-MYD contains a sequence from the MyD88 TIR homodimerization domain (RDVLPGT)¹ preceded by a protein transduction sequence (RQIKIWFQNRRMKWKK) derived from antennapedia which enables the peptide to translocate through the cell membrane². Pepinh-MYD is provided with Pepinh-Control, a control peptide³.

1. **Loiarro M. et al., 2005.** Peptide-mediated Interference of TIR Domain Dimerization in MyD88 Inhibits Interleukin-1-dependent Activation of NF- κ B. *J. Biol. Chem.*, 280: 15809-14.
2. **Derossi D. et al., 1994.** The third helix of the Antennapedia homeodomain translocates through biological membranes. *J. Biol. Chem.*, 269: 10444-50.
3. **Toshchakov VU. et al., 2005.** Differential Involvement of BB Loops of Toll-IL-1 Resistance (TIR) Domain-Containing Adapter Proteins in TLR4- versus TLR2-Mediated Signal Transduction. *J. Immunol.*, 175: 494-500.

Species reactivity: Human, mouse

Sequences:

- Pepinh-MYD: RQIKIWFQNRRMKWKK-RDVLPGTVCVNS-NH₂ (MyD88 homodimerization sequence is underlined)
- Pepinh-Control: RQIKIWFQNRRMKWKK-SLHGRGDPMEAFII-NH₂

Molecular weights:

- Pepinh-MYD: 3430
- Pepinh-Control: 3812

Purity: \geq 95% (UHPLC)

Working concentration: 5-50 μ M

METHODS

Preparation of 1 mM stock solutions:

Note: Spin briefly the vial before opening the cap. Bring the endotoxin-free water (provided) at room temperature.

- Pepinh-MYD:
 - Add 588 μ l of endotoxin-free water to the vial and mix by vortexing.
 - Pepinh-Control:
 - Add 525 μ l of endotoxin-free water to the vial and mix by vortexing.
- Note:* Further dilutions can be prepared using endotoxin-free water or PBS.

Inhibition of MyD88:

The following protocol describes the inhibition of MyD88 in THP1-Blue™-CD14 cells. THP1-Blue™-CD14 cells derive from the human monocytic cell line THP-1 and overexpress the CD14 gene to enhance their response to lipopolysaccharide (LPS). They induce NF- κ B in response to ligands for TLR2, TLR4, TLR5 and TLR8, all four TLRs signaling through MyD88. NF- κ B activation can be readily assessed in these cells as they stably express an NF- κ B-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene.

- 1- Pretreat THP1-Blue™-CD14 cells with Pepinh-MYD or Pepinh-Control at 5-50 μ M for 6 hours at 37°C.
- 2- Add increasing concentrations of TLR2 (Pam3CSK4), TLR4 (LPS), TLR5 (flagellin) or TLR8 (R848) ligands.
- 3- Incubate at 37°C overnight and collect supernatants.
- 4- Determine NF- κ B activation by assessing SEAP present in the supernatants using QUANTI-Blue™, a SEAP detection medium. Simply add 20 μ l of each supernatant to 180 μ l of reconstituted QUANTI-Blue™ and incubate for 0.5-6 hours. Determine SEAP activity spectrophotometrically at 620-655 nm.

RELATED PRODUCTS

Product	Catalog Code
Pam3CSK4	tlrl-pms
LPS-EK (lipopolysaccharide from <i>E. coli</i> K12)	tlrl-eklps
FLA-ST ultrapure (flagellin from <i>S. typhimurium</i>)	tlrl-pstfla
R848	tlrl-r848
QUANTI-Blue™	rep-qb1

TECHNICAL SUPPORT

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