

Pepinh-TRIF

TRIF Inhibitory Peptide

Catalog code: tlrl-pitrif

<http://www.invivogen.com/pepinh-trif>

For research use only

Version # 17L13-MM

PRODUCT INFORMATION

Contents

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

Storage and stability

- Pepinh-TRIF and Pepinh-Control are provided lyophilized and shipped at room temperature. Store at -20°C. Lyophilized product is stable for 1 year at -20°C.

- Upon resuspension, prepare aliquots of Pepinh-TRIF and Pepinh-Control and store at -20°C. Avoid repeated freeze-thaw cycles. Resuspended product is stable for 6-8 months at -20°C when properly stored.

DESCRIPTION

Pepinh-TRIF is a 30 aa peptide that blocks TRIF signaling by interfering with TLR-TRIF interaction. Pepinh-TRIF contains the 14 aa that correspond to the sequence of the BB loop of TRIF (FCEEFQVPGRGELH)¹ linked to the cell-penetrating segment of the antennapedia homeodomain (RQIKIWFQNRRMKWKK)². Pepinh-TRIF is provided with Pepinh-Control, a control peptide¹.

1. Tshchakov 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. 2. Derossi D. *et al.*, 1994. The third helix of the Antennapedia homeodomain translocates through biological membranes. *J. Biol. Chem.*, 269: 10444-50.

Species reactivity: Human, mouse

Sequences:

- Pepinh-TRIF: RQIKIWFQNRRMKWKK-FCEEFQVPGRGELH-NH₂ (TRIF homodimerization sequence is underlined)
- Pepinh-Control: RQIKIWFQNRRMKWKK-SLHGRGDPMEAFII-NH₂

Molecular weights:

- Pepinh-TRIF: 3918
- Pepinh-Control: 3812

Purity: ≥ 95% (UHPLC)

Working concentration: 5-50 μM

METHODS

Preparation of 1 mM stock solution

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

- Pepinh-TRIF:
 - Add 510 μl of endotoxin-free water to the vial and mix by vortexing.

- Pepinh-Control:
 - Add 525 μl 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 TRIF

The following protocol describes the inhibition of TRIF in Ramos-Blue™ cells. Ramos-Blue™ cells are B lymphocytes that stably express an NF-κB/AP-1-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. They induce NF-κB in response to various ligands, including poly(I:C) a ligand for TLR3 which signals exclusively through TRIF. NF-κB activation can be readily assessed in these cells by monitoring the production of SEAP.

1. Pretreat Ramos-Blue™ cells with Pepinh-TRIF or Pepinh-Control at 5-50 μM for 6 hours at 37°C.
2. Add increasing concentrations of poly(I:C).
3. Incubate at 37°C overnight and collect supernatants.
4. Determine NF-κB activation by assessing SEAP present in the supernatants using QUANTI-Blue™, a SEAP detection medium. Simply add 20 μl of each supernatant to 180 μl of reconstituted QUANTI-Blue™ and incubate for 0.5-6 hours. Determine SEAP activity spectrophotometrically at 620-655 nm.

RELATED PRODUCTS

Product	Catalog Code
Ramos-Blue™	rms-sp
Poly(I:C)	tlrl-pic
QUANTI-Blue™	rep-qb1

TECHNICAL SUPPORT

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