

# Product Datasheet

## TLR4 Inhibitor Peptide Set

### NBP2-26244

Unit Size: 2 Vials

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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**NBP2-26244**

## TLR4 Inhibitor Peptide Set

Product Information	
<b>Unit Size</b>	2 Vials
<b>Concentration</b>	Lyoph
<b>Storage</b>	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
<b>Reconstitution Instructions</b>	Please contact technical support for detailed reconstitution instructions.
<b>Purity</b>	>95%, by HPLC.
Product Description	
<b>Description</b>	TLR4 inhibitor and control peptides are quality controlled in vitro using the TLR4/MD-2/CD14/NF-kB/SEAP cell line with SEAP as a readout assay (Image 3).
<b>Gene ID</b>	7099
<b>Gene Symbol</b>	TLR4
<b>Species</b>	Human, Mouse, Rat
<b>Reactivity Notes</b>	Rat reactivity reported in scientific literature (PMID: 25811788)
<b>Preparation Method</b>	<p>Preparation of 5 mM VIPER and CP7 Stock Solutions            Note: Bring the peptides to room temperature and quick spin the tubes before opening the caps.</p> <p>VIPER: A final volume of 72 ul will make a 5 mM stock solution. Add 72 ul sterile H2O to the tube of peptide. Carefully pipet to ensure all of the peptide is dissolved.</p> <p>CP7: A final volume of 76 ul will make a 5 mM stock solution. Add 76 ul sterile H2O to the tube of peptide. Carefully pipet to ensure all of the peptide is dissolved.</p> <p>The stock solutions may be diluted further to make working solutions. Dilute according to the needs for your assay. For example dilute 5 mM stock solutions 1:10 in sterile 1X PBS or cell culture media to make 500 uM working solutions. Working solutions should be made fresh daily and not stored.</p>
<b>Inhibitor Family</b>	TLR
<b>Inhibitor Target</b>	TLR4
<b>Inhibitor Content</b>	<p>VIPER: A TLR4 Inhibitory Peptide: 1 mg (lyophilized)            KYSEFKLILAEYRRRRRRRRR (VIPER sequence: KYSEFKLILAEY). Molecular weight: 2780.3</p> <p>CP7, Control Peptide: 1 mg (lyophilized) RNTISGNIYSARRRRRRRRR (Control sequence: RNTISGNIYSA). Molecular weight: 2601</p>
Product Application Details	
<b>Applications</b>	Flow Cytometry, Functional, In vitro assay, Block/Neutralize, Binding Inhibition
<b>Recommended Dilutions</b>	Flow Cytometry, Functional reported in scientific literature (PMID 24630524), In vitro assay, Binding Inhibition reported in scientific literature (PMID 27897392), Block/Neutralize reported in multiple pieces of scientific literature

**Application Notes**

The inhibitor is used in assays to inhibit TLR4 activation; see Image 3 and also refer to Lysakova-Devine et al (2010) for examples. Optimal inhibitor concentrations should be established through titration and may vary between model systems. We recommend an initial titration from 0-30 uM for in vitro assays (Image 3). Control concentrations should 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 peptides for 2 h prior to TLR4 activation with LPS (Image 3); however, optimal preincubation times may vary between model systems.

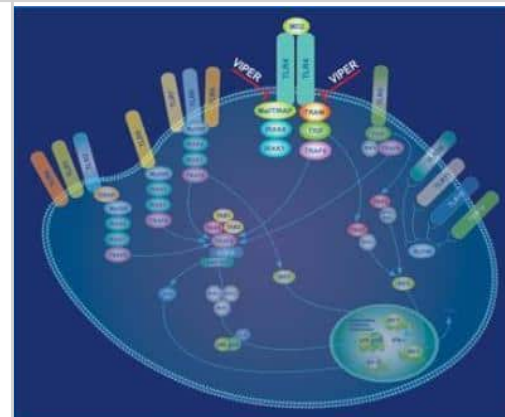
The TLR4/MD-2/CD14 stably transfected cell line is a useful positive control model system for studying inhibition of TLR4 activation by VIPER (Image 3). SEAP is used as a readout assay in Image 3 to measure TLR4 inhibition.

A novel model system is shown in Image 1 where TLR4 inhibitor peptide, but not CP7, inhibited TLR4 activation in Mal-deficient immortalized mouse bone marrow-derived macrophages (iBMDMs). In these iBMDMs, the inhibitor targets TLR4-TRAM, but not TLR-Mal, interactions as Mal is not expressed. TNF-alpha is used as a readout assay in Image 1 to measure inhibition.

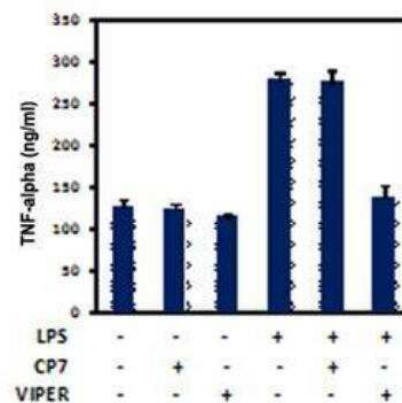


## Images

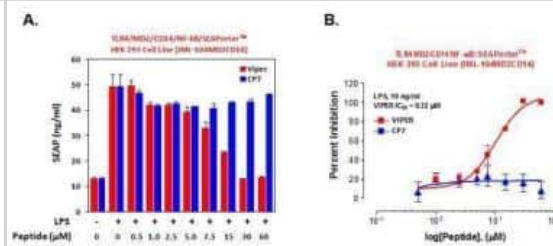
TLR4 Inhibitor Peptide Set [NBP2-26244] - The inhibitor peptide inhibits TLR4 signaling by blocking interactions between TLR4 and its adaptors Mal/TIRAP and TRAM.



Blocking/Neutralizing: TLR4 Inhibitor Peptide Set [NBP2-26244] - iBMDM cultures were pretreated with 5uM of inhibitor or control prior to stimulation with 20 ng/ml LPS. Negative (LPS-, LPS-/CP7+, LPS-/CP7+) and positive (LPS+) controls were also included. Supernatants were harvested after 6 h and murine TNF-alpha measured by ELISA. TLR4 inhibitor, but not the control, inhibited LPS-induced TNF-alpha production. Image courtesy of Dr. Julianne Stack and Dr. Andrew Bowie, Trinity College, Dublin, Ireland.



TLR4 Inhibitor Peptide Set [NBP2-26244] - TLR4/MD-2/CD14/NF-kB/SEAPorter stably transfected cells were plated in 96-well plates at  $1 \times 10^5$  cells/well. After 16 h, cells were preincubated with various concentrations of inhibitor or control peptides (CP) for 1 h. Cells were then stimulated with 10 ng/ml LPS for 24 h. The secreted alkaline phosphatase (SEAP) was analyzed using the SEAPorter Assay Kit. Inhibitory effect on the LPS-mediated TLR4 activation by the VIPER peptide is shown in panel A, and IC<sub>50</sub> of the VIPER peptide is described in panel B.



## Publications

Verma S, Reddy P, Sowdhamini R Integrated approaches for the recognition of small molecule inhibitors for Toll-like receptor 4 Computational and Structural Biotechnology Journal 2023-07-22 [PMID: 37576745] (In vitro)

Yang D, Dai X, Xing Y et al. Intrinsic cardiac adrenergic cells contribute to LPS-induced myocardial dysfunction Communications biology 2022-01-25 [PMID: 35079095]

Yamamoto K, Kondo Y, Ohnishi S Et al. The TLR4-TRIF-type 1 IFN-IFN- gamma pathway is crucial for gastric MALT lymphoma formation after Helicobacter suis infection iScience 2021-08-01 [PMID: 34585114] (In vitro)

Gergen AK, Jarrett MJ, Li A et al. Toll-like Receptor 4 Mediates Reflux-Induced Inflammation in a Murine Reflux Model Seminars in thoracic and cardiovascular surgery 2021-09-14 [PMID: 34534678]

Mizuno Y, Taguchi T Self-assembled dodecyl group-modified gelatin microparticle-based hydrogels with angiogenic properties NPG Asia Mater 2020-12-01

Li B, Yan C, Wu J et al. Clonorchis sinensis ESPs enhance the activation of hepatic stellate cells by a cross-talk of TLR4 and TGF-beta/Smads Signaling pathway Acta Trop. 2019-12-17 [PMID: 31862462] (Func)

Harada Y, Zhang J, Imari K et al. Cathepsin E in neutrophils contributes to the generation of neuropathic pain in experimental autoimmune encephalomyelitis Pain 2019-09-01 [PMID: 31095099] (Mouse)

Nishida M, Saegusa J, Tanaka S, Morinobu A. S100A12 facilitates osteoclast differentiation from human monocytes. PLoS ONE 2018-09-20 [PMID: 30235276] (Human)

Chehimi M, Ward R, Pestel J et al. Omega-3 Polyunsaturated Fatty Acids Inhibit IL-17A Secretion through Decreased ICAM-1 Expression in T Cells Co-Cultured with Adipose-Derived Stem Cells Harvested from Adipose Tissues of Obese Subjects Mol Nutr Food Res 2019-03-08 [PMID: 30848861] (B/N, Human)

Tun X, Yasukawa K, Yamada KI. Nitric Oxide Is Involved in Activation of Toll-Like Receptor 4 Signaling through Tyrosine Nitration of Src Homology Protein Tyrosine Phosphatase 2 in Murine Dextran Sulfate-Induced Colitis Biol. Pharm. Bull. 2018-12-04 [PMID: 30504685] (B/N, Mouse)

Sahoo SS, Pratheek BM, Meena VS et al. VIPER regulates naive T cell activation and effector responses: Implication in TLR4 associated acute stage T cell responses Sci Rep 2018-05-08 [PMID: 29740052] (B/N, Mouse)

Inoue H, Shirakawa J, Togashi Y et al. Signaling between pancreatic B-cells and macrophages via S100 calcium-binding protein A8 exacerbates B-cell apoptosis and islet inflammation. J. Biol. Chem. 2018-03-01 [PMID: 29496993] (B/N)

More publications at <http://www.novusbio.com/NBP2-26244>

## Procedures

### MSDS (NBP2-26244)

[[URL:https://www.novusbio.com/products/tlr4-inhibitor\_nbp2-26244]][[Caption:TLR4 Inhibitor Peptide Set]]

#### Hazard Information

Chemical Name: Non hazardous products

Chemical Formula: N/A

CAS Number: N/A

EEC-No: N/A

#### Hazard Identification

None

#### First Aid Measures

Eye Contact: None

Skin Contact: None

Inhalation: None

Ingestion: None

#### Accidental Release Measures

This product either does not contain hazardous constituents or the concentration of all chemical constituents are below the regulatory threshold limits described by Occupational Safety Health Administration Hazard Communication Standard 29 CFR 1910.1200 and the European Directive 91/155/EEC, 88/379/EEC, and 67/546/EEC.

#### Handling and Storage

Exposure Controls / Personal Protection

Other Precautions: None

#### Physical and Chemical Properties

Form: N/A

Color: N/A

Odor: N/A

Melting Point: N/A

Boiling Temperature: N/A

Density: N/A

Vapor Pressure: N/A

Solubility in Water: N/A

Flash Point: N/A

Explosion limits: N/A

Ignition Temperature: N/A

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### **Products Related to NBP2-26244**

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NBP2-24821PEP	TLR4 Antibody Blocking Peptide
210-TA-005	TNF-alpha [Unconjugated]
NB100-56566	TLR4 Antibody (76B357.1) - BSA Free
D6050	IL-6 [HRP]

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### **Limitations**

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Inhibitors are guaranteed for 1 year from date of receipt.

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