

# LL-37

## Human antimicrobial peptide with immunomodulatory properties

Catalog # tlr1-l37

For research use only

Version # 16C24-MM

### PRODUCT INFORMATION

#### Content:

- 1 mg synthetic LL-37
- 1.5 ml endotoxin-free water

#### Storage and stability:

- LL-37 is provided lyophilized and shipped at room temperature. Store at -20°C. Lyophilized product is stable for 2 years when properly stored.
- Upon resuspension, prepare aliquots of LL-37 and store at -20°C or -80°C. Avoid repeated freeze-thaw cycles. Resuspended product is stable for 1 month at -20°C when properly stored.

### DESCRIPTION

LL-37, also known as hCAP18, is the C-terminal part of the only human cathelicidin identified to date called human cationic antimicrobial protein (hCAP). This antimicrobial peptide is referred to as LL-37 as it has a 37 amino acid sequence starting with two leucines. It exhibits a variety of immunomodulatory functions such as bactericidal action, chemotaxis, activation of chemokine secretion and antiseptic effect<sup>1</sup>. This synthetic peptide has been shown to suppress the inflammatory response induced by lipopolysaccharide (LPS) and other Toll-like Receptors (TLR) ligands<sup>2</sup>. Conversely, it can enhance TLR3 signaling by interacting directly with dsRNA, such as poly(I:C)<sup>3</sup>. Furthermore LL-37 can form a complex with ssDNA to trigger TLR9<sup>4</sup> and with ssRNA to trigger TLR7 and TLR8<sup>5</sup>. Moreover, it exhibits anti-inflammatory activity by binding to DNA in the cytosol and inhibiting AIM2 inflammasome formation<sup>6</sup>. In general the physiological role of LL-37 is protective, however, its overexpression is associated with auto-immunity.

1. **Scott A. et al., 2011.** Evaluation of the ability of LL-37 to neutralise LPS *in vitro* and *ex vivo*. *PLoS One.* 6(10): e26525. 2. **Di Nardo A. et al., 2007.** Cathelicidin Antimicrobial Peptides Block Dendritic Cell TLR4 Activation and Allergic Contact Sensitization. *J. Immunol.* 178: 1829 - 1834. 3. **Lai Y. et al., 2011.** LL37 and cationic peptides enhance TLR3 signaling by viral double-stranded RNAs. *PLoS One.* 6(10):e26632. 4. **Lande R et al., 2007.** Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature.* 449(7162):564-9. 5. **Ganguly D. et al., 2009.** Self-RNA-antimicrobial peptide complexes activate human dendritic cells through TLR7 and TLR8. *J Exp Med.* 206(9):1983-94. 6. **Dombrowski Y. et al., 2011.** Cytosolic DNA triggers inflammasome activation in keratinocytes in psoriatic lesions. *Sci Transl Med.* 11;3(82):82ra38.

### CHEMICAL PROPERTIES

**Solubility:** Water (1 mg/ml)

#### Amino acid sequence:

LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLPRTES

**Molecular formula:** C<sub>205</sub>H<sub>340</sub>N<sub>60</sub>O<sub>35</sub>

**Molecular weight:** 4493.37

**Purity:** ≥ 95 % (HPLC)

### METHODS

#### Preparation of LL-37 stock solution (1 mg/ml)

1. Add 1 ml endotoxin-free water to 1 mg LL-37 vial.
2. Vortex until completely dissolved.
3. Prepare aliquots and store stock solution at -20°C.

### APPLICATIONS

Described below is one application for LL-37. Other applications have not been tested.

#### **LPS neutralization in THP1-XBlue™ MD2-CD14 cells**

THP1-XBlue™ MD2-CD14 cells derive from THP-1 cells, a human monocytic cell line that naturally expresses most TLRs. THP1-XBlue™ MD2-CD14 cells express an NF-κB/AP-1 inducible secreted embryonic alkaline phosphatase (SEAP) gene, and overexpress the MD2 and CD14 genes. Overexpression of MD2 and CD14 increases the TLR4 response. Upon TLR stimulation and subsequent NF-κB/AP-1 activation, THP1-XBlue™ MD2-CD14 cells secrete SEAP, which is easily detectable using QUANTI-Blue™, a medium that turns purple/blue in the presence of SEAP.

- Prepare a cell suspension at 625,000 cells/ml in RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum.
- Add 160 µl of THP1-XBlue™ MD2-CD14 cell suspension per well of a 96-well plate (~ 100,000 cells/well).
- Add 20 µl of LPS-EB Ultrapure (1- 100 ng/ml final concentration).
- Add 20 µl of LL-37 (1-100 µg/ml final concentration).
- Incubate 16-24 hours at 37°C in 5% CO<sub>2</sub>.
- Add 20 µl supernatant to 180 µl QUANTI-Blue™ in a 96-well plate.
- Incubate 1-3 hours at 37°C
- Assess SEAP levels with the naked eye or spectrophotometrically by reading the OD at 655 nm.

### RELATED PRODUCTS

Product	Catalog Code
THP1-XBlue™ MD2-CD14 cells	thpx-mdcdsp
LPS-EB Ultrapure	tlr1-3pelps
QUANTI-Blue™	rep-qb1
Poly(I:C)	tlr1-pic

#### TECHNICAL SUPPORT

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