# **CL531**

# Dual TLR2 & TLR7 ligand

Catalog # tlrl-c531

For research use only

Version # 15B04-MM

#### PRODUCT INFORMATION

#### **Content:**

- 500 µg CL531 provided as a lyophilized powder
- 1.5 ml endotoxin-free water

#### Storage:

- CL531 is shipped at room temperature. Store lyophilized product at -20 °C. Lyophilized product is stable for 1 year at -20 °C.
- Upon resuspension, store at 4 °C. Resuspended product is stable for 6 months at 4 °C. Do not store resuspended product in plastic tubes.

### **DESCRIPTION**

CL531 is an 8-hydroxyadenine derivative conjugated to the lateral chain of the second lysine of Pam2CSK4. CL531 is a very potent TLR2 agonist and a good TLR7 agonist. TLR2-mediated activation of NF-κB is achieved with concentrations as low as 5 pM (0.01 ng/ml).

### **APPLICATIONS**

CL531 can be used to stimulate both TLR2 and TLR7.

## **BACKGROUND**

InvivoGen has developed a series of novel molecules designed to induce potent immune responses through the combined activation of several pattern recognition receptors (PRRs) that trigger different innate immune signaling pathways. These molecules are agonists for TLR2, TLR7 or both. Agonists that activate TLR2 are derived from the well-established TLR2 ligand, Pam2CSK4, and those recognized by TLR7 are derived from the 8-hydroxyadenine derivative CL264, a TLR7 agonist recently developed by InvivoGen (see Related Products overleaf).

TLR2 and TLR7 are two PRRs with distinct characteristics. TLR2 is a cell surface receptor expressed by many cell types, while TLR7 is an endosomal receptor expressed predominantly in plasmacytoid dendritic cells (pDC) and to a lesser extent in B cells. TLR2 signaling triggers the NF- $\kappa$ B pathway and the production of pro-inflammatory cytokines, such as TNF- $\alpha$ , whereas TLR7 signaling induces mainly the IRF pathway and the production of IFN- $\alpha$ . Combined activation of these different pathways results in robust immune responses with potential therapeutic effects. InvivoGen's multi-PRR agonists are promising candidates for antitumor and vaccine applications.

## **CHEMICAL PROPERTIES**

**Synonym:** S-(2,3-bis(palmitoyloxy)-(2RS)propyl)-(R)-cysteinyl-(S)-seryl-(S)-lysyl-Ne-(4-((6-amino-2-(butylamino)-8-hydroxy-9H-purin-9-yl)methyl) benzylamido)(S)-lysyl-(S)-lysyl-(S)-lysine

Formula: C82H144N16O14S Molecular weight: 1610 g/mol Solubility: H2O (1 mg/ml)

Working concentration: 5 pg - 10 μg/ml (~3 pM - 10 μM)

Endotoxin level: <0.001 EU/µg

**Structure:** 

## **METHODS**

### Preparation of CL531 stock solution (1 mg/ml)

- Add 500  $\mu l$  water to 500  $\mu g$  CL531. Vortex until complete solubilization.

#### TLR stimulation with CL531 using HEK-Blue cells

CL531 can be used to stimulate TLR2 in HEK-Blue™ TLR2 cells and TLR7 in HEK-Blue™ TLR7 cells. These cells stably express an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) and overexpress the appropriate TLR gene. For more information visit: www.invivogen.com/hek-blue-cells

- 1. Stimulate HEK-Blue™ TLR2 cells with 5 pg 50 ng/ml CL531 and HEK-Blue™ TLR7 cells with 100 ng 10 µg/ml CL531.
- 2. Incubate for 6 24 h at 37 °C, 5% CO2.
- 3. Determine TLR stimulation using a SEAP detection medium, such as QUANTI-Blue™ or HEK-Blue™ Detection (see Related Products, overleaf) or by assessing cytokine expression using an ELISA.



## RELATED PRODUCTS

| Product  | Catalog Code |
|--|--------------|
| HEK-Blue™ hTLR2 Cells  | hkb-htlr2    |
| HEK-Blue™ mTLR2 Cells  | hkb-mtlr2    |
| HEK-Blue™ hTLR7 Cells  | hkb-htlr7    |
| HEK-Blue™ mTLR7 Cells  | hkb-mtlr7    |
| RAW-Blue™ Cells (Mouse macrophage reporter cells)                | raw-sp       |
| HEK-Blue™ Detection (SEAP detection medium)                      | hb-det2      |
| QUANTI-Blue™ (SEAP detection medium)                             | rep-qb1      |
| Dual TLR2 & TLR7 ligands:  |              |
| Adilipoline™ (CL413; TLR2 & TLR7 ligand)                         | tlrl-c413    |
| CL401 (TLR2 & TLR7 ligand)                                       | tlrl-c401    |
| CL572 (TLR2 (human) & TLR7 ligand)                               | tlrl-c572    |
| TLR ligands & nucleic acid carriers:                             |              |
| AdiFectin™ (CL347; TLR7 ligand & nucleic acid carrier)           | tlrl-c347    |
| CL419 (TLR2 ligand & nucleic acid carrier)                       | tlrl-c419    |
| PamadiFectin™ (CL553; TLR7 & TLR2 ligand & nucleic acid carrier) | tlrl-c553    |
| TLR2 ligands:  |              |
| HKLM (Heat killed Listeria monocytogenes)                        | tlrl-hklm    |
| FSL-1 (Synthetic diacylated lipoprotein)                         | tlrl-fsl     |
| Pam2CSK4 (Synthetic diacylated lipoprotein)                      | tlrl-pm2s    |
| TLR7 ligands:  |              |
| CL264 (Adenine analog)   | tlrl-c264e   |
| CL307 (Spermine covalently linked to CL264)                      | tlrl-c307    |
| Gardiquimod™ (Imidazoquinoline compound)                         | tlrl-gdqs    |

