

PamadiFectin™ (CL553)

TLR2 & TLR7 ligand and a nucleic acid carrier

Catalog # tlr1-c553

For research use only

Version # 15B04-MM

PRODUCT INFORMATION

Content:

- 500 µg PamadiFectin™ (CL553) provided as a lyophilized powder
- 1.5 ml endotoxin-free water

Storage:

- PamadiFectin™ 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 6 months at 4 °C. Do not store resuspended product in plastic tubes.

DESCRIPTION

PamadiFectin™ was generated by conjugation of the potent TLR7 ligand CL307 to a Pam2C group. PamadiFectin™ induces NF-κB activation through stimulation of both TLR2 and TLR7. In addition, at physiological pH, PamadiFectin™ is able to form complexes with nucleic acids and carry them in the cytosol and nucleus leading to a strong induction of the interferon regulatory factor (IRF) pathway. Intratumoral administration of pDNA/PamadiFectin™ complexes leads to spectacular reduction of tumor growth and improved long-term survival in B16-F1 tumor-bearing mice. Tumor rechallenge experiments have not yet been performed.

APPLICATIONS

PamadiFectin™ can be used to stimulate TLR2 and TLR7 or as a nucleic acid carrier (described overleaf).

CHEMICAL PROPERTIES

Synonym: N4-(S-((2,3-bis(palmitoyloxy))-(2R)propyl)-(R)-cysteinyl) N1-(4-(((6-amino-2-(butylamino)-8-hydroxy-9H-purin-9-yl)methyl)benzoyl) glycinyl) spermine

Formula: C₆₇H₁₁₈N₁₂O₈S

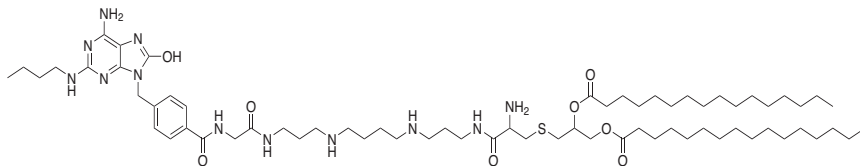
Molecular weight: 1251 g/mol

Solubility: Water (1 mg/ml)

Working concentration: 100 ng - 1 µg/ml (~100 nM - 1 µM)

Endotoxin level: <0.001 EU/µg

Structure:



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-κB pathway and the production of pro-inflammatory cytokines, such as TNF-α, whereas TLR7 signaling induces mainly the IRF pathway and the production of IFN-α. 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.

METHODS

PamadiFectin™ as TLR2 and TLR7 ligand

Preparation of PamadiFectin stock solution (1 mg/ml)

1. Add 500 µl endotoxin-free water (provided) to 500 µg PamadiFectin™. Vortex until complete solubilization.

TLR stimulation with PamadiFectin using HEK-Blue cells

PamadiFectin™ 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™ TLR cells with 100 ng - 1 µg/ml PamadiFectin™.
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.

TECHNICAL SUPPORT

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PamadiFectin™ as a nucleic acid carrier

PamadiFectin™ has the ability to form complexes with nucleic acids (for example, double-stranded DNA, such as short oligonucleotides or plasmid DNA, or single-stranded RNA) and facilitate their penetration into the cell resulting in their recognition by additional PRRs that sense nucleic acids (e.g. the cytosolic DNA sensors DDX41 and IFI16 and the dsRNA receptors TLR3 and RIG-I/MDA-5) leading mainly to the induction of type I interferons (IFNs). The ability of PamadiFectin™ to complex nucleic acids is conferred by the presence of a cationic lipid.

Preparation of Nucleic Acid / PamadiFectin complexes

1. Resuspend PamadiFectin™ at a concentration of 1 mg/ml, as described overleaf.
2. Dilute 6 µl of PamadiFectin™ with 94 µl serum-free culture medium. Vortex gently 1-2 seconds to mix and incubate for 5-10 min at room temperature.
3. In a sterile 1.5 ml microfuge tube, add 100 µl of the diluted PamadiFectin™ to 1µg nucleic acid, such as plasmid DNA, a RIG-I ligand or a cytosolic DNA sensor (CDS) ligand. Tap gently to mix.
4. Incubate at room temperature for 10-20 minutes to allow the formation of the complex. Do not store complex for more than 1 day.

Induction of type I IFNs with Nucleic Acid / PamadiFectin™

Induction of type I IFNs with intracellular nucleic acids can be studied in a variety of cells. In order to facilitate the study of type I IFN production and the induction of interferon stimulated genes (ISG) through interferon regulatory factors (IRFs), InvivoGen has developed stable reporter cells in two well established immune cell models, the human monocytic THP-1 cell line and the murine RAW 264.7 macrophages.

Induction of type I IFNs in THP1-Blue ISG cells

THP1-Blue™ ISG cells were derived from the human THP-1 monocyte cell line by stable integration of an IRF-inducible SEAP reporter construct. THP1-Blue™ ISG cells are highly responsive to PRR agonists that trigger the IFN signaling pathway, such as transfected double-stranded nucleic acid. A protocol for the induction of type I IFNs using THP1-Blue™ ISG cells is given below:

1. Prepare nucleic acid / PamadiFectin™ complex, as described above.
2. Treat cells with nucleic acid / PamadiFectin™ complex, for example 6 µg PamadiFectin™ complexed with 1µg of HSV-60 (see Related Products), for 18 - 24 hours.
3. Monitor induction of type I IFNs by measuring the levels of IRF-induced SEAP in the cell culture supernatant using QUANTI-Blue™, a SEAP detection reagent.

RELATED PRODUCTS

Product	Catalog Code
Reporter Cells	
HEK-Blue™ hTLR7 Cells	hkb-htr7
HEK-Blue™ mTLR7 Cells	hkb-mtlr7
RAW-Blue™ Cells (Mouse macrophage reporter cells)	raw-sp
Raw-Blue™ ISG Cells (IRF reporter macrophages)	raw-isg
THP1-Blue™ ISG Cells (Human IRF reporter monocytes)	thp-isg
THP1-Blue™ ISG-KD STING Cells (STING knockdown)	thp-kdstg
SEAP Detection Media	
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
CL531 (TLR2 & TLR7 ligand)	tlrl-c531
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™ (TLR7 & TLR2 ligand, nucleic acid carrier)	tlrl-c553
TLR2 ligands:	
HKLM (Heat killed <i>Listeria monocytogenes</i>)	tlrl-hklm
FSL-1 (Synthetic diacylated lipoprotein)	tlrl-fsl
Pam2CSK4 (Synthetic diacylated lipoprotein)	tlrl-pm2s
TLR7 ligands:	
CL264 (8-hydroxyadenine derivative)	tlrl-c264e
CL307 (Spermine covalently linked to CL264)	tlrl-c307
Gardiquimod™ (Imidazoquinoline compound)	tlrl-gdq
CDS & RIG-I ligands	
HSV-60 Naked (CDS ligand)	tlrl-hsv60n
ISD Naked (CDS ligand)	tlrl-isdn
pCpGfree-giant Naked (CDS ligand)	tlrl-cpgfn
poly(dA:dT) Naked (RIG-I ligand - CDS ligand)	tlrl-patn
poly(dG:dC) Naked (RIG-I ligand - CDS ligand)	tlrl-pgen
VACV-70 Naked (CDS ligand)	tlrl-vav70n
5'ppp-dsRNA (RIG-I ligand)	ttlrl-3prna

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