OxPAPC

TLR2 and TLR4 Inhibitor

Catalog code: tlrl-oxp1

https://www.invivogen.com/oxpapc

For research use only

Version 22E13-MM

PRODUCT INFORMATION

Contents

- 2 x 0.5 mg OxPAPC provided as a transparent film.

Storage and stability

- Product is shipped at room temperature. Upon receipt, store at -20°C.
- Aliquots of OxPAPC lipidic film following resuspension and evaporation of chloroform can be stored at -70°C for 3 months. Avoid repeated freeze-thaw cycles.
- Do **not** store OxPAPC resuspended in culture medium.

Quality control

- The inhibitory activity has been validated using cellular assays.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

OxPAPC, a TLR2 and TLR4 inhibitor, is a bioactive principal component of minimally modified low-density lipoprotein (MM-LDL). It is generated by the oxidation of 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphorylcholine (PAPC), a major component of mammalian cell membranes that becomes oxidized by reactive oxygen species (ROS) released from dead or dying cells[1]. The oxidation of PAPC results in a mixture of oxidized phospholipids containing either fragmented or full length oxygenated sn-2 residues. Both fragmented and full-length oxygenated species can modulate inflammatory responses. Oxidized phospholipids play important roles in multiple physiological and pathophysiological conditions[1].

OxPAPC has been shown to inhibit the signaling induced by bacterial lipopeptides and lipopolysaccharide (LPS). It acts by competing with LPS-binding protein (LBP), CD14 and, myeloid differentiation protein 2 (MD-2), the accessory proteins that interact with bacterial lipids, thus blocking the signaling of TLR2 and TLR4[2, 3]. Interestingly, it has been suggested that in animal models OxPAPC protects from septic shock by targeting the non-canonical inflammasome in macrophages[4].

1. Stamenkovic A. *et al.*, 2019. Oxidized lipids: not just another brick in the wall. Can J Physiol Pharmacol. 97(6):473-85. 2. Erridge C. *et al.*, 2008. Oxidized phospholipid inhibition of Toll-like receptor (TLR) signaling is restricted to TLR2 and TLR4: roles for CD14, LPS-binding protein, and MD2 as targets for specificity of inhibition. J. Biol. Chem., 283:24748-59. 3. von Schlieffen E. *et al.*, 2009. Multi-hit inhibition of circulating and cell-associated components of the Toll-like receptor 4 pathway by oxidized phospholipids. Arterioscler Thromb Vasc Biol, 29: 356-62. 4. Chu L.H. *et al.*, 2018. The oxidized phospholipid oxPAPC protects from septic shock by targeting the non-canonical inflammasome in macrophages. Nat Commun. 9(1):996.

PRODUCT RESUSPENSION

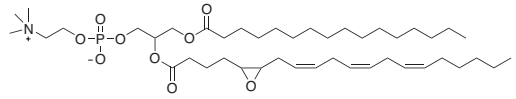
For use of total amount:

Add 500 µl of culture medium (containing 10% heat-inactivated fetal calf serum) to the vial to obtain a lipid concentration of 1 mg/ml. Resuspend OxPAPC by vigorous vortexing for at least 30 seconds. Warm the vial up to 30°C and vortex again for 1 minute. Avoid preparing concentrated stocks as OxPAPC is poorly soluble in water. High concentrations of OxPAPC can be toxic. Use the resuspended OxPAPC immediately, as oxidized phospholipids are inherently unstable. **DO NOT STORE RESUSPENDED PRODUCT.**

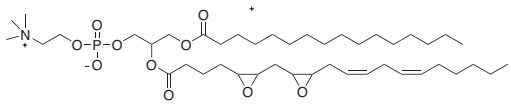
Alternatively for use of partial amount:

If you wish to use a partial amount of the product, follow the procedure described here to conserve the product activity. Add 500 µl of chloroform to the vial to obtain a lipid concentration of 1 mg/ml and carefully vortex avoiding contact of the solvent with the vial cap. Prepare aliquots of OxPAPC solution in sterile glass (optimal) or polypropylene cell culture tubes. Before use check if the tubes are resistant to chloroform. Evaporate chloroform under a stream of nitrogen or argon gas with simultaneous vortexing in order to obtain a thin film of lipid on the tube walls. The OxPAPC lipidic film can be stored at -70°C for 3 months. Avoid repeated freeze-thaw cycles. Resuspend OxPAPC lipidic film in culture medium as described above.

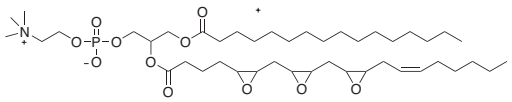
CHEMICAL PROPERTIES



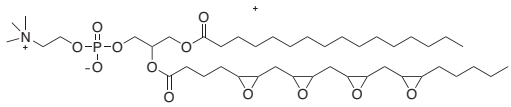
Chemical Formula: $C_{44}H_{80}NO_9P$
Molecular Weight: 798.08 g/mol



Chemical Formula: $C_{44}H_{80}NO_{10}P$
Molecular Weight: 814.08 g/mol



Chemical Formula: $C_{44}H_{80}NO_{11}P$
Molecular Weight: 830.08 g/mol



Chemical Formula: $C_{44}H_{80}NO_{12}P$
Molecular Weight: 846.08 g/mol

TECHNICAL SUPPORT
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METHODS

Working concentration: 30 µg/ml

TLR2/TLR4 inhibition

To assess the role of TLR2/TLR4, the appropriate TLR ligand with or without OxPAPC (30 µg/ml) was added to HEK-Blue™ hTLR2 or HEK-Blue™ hTLR4 cells, and then incubated at 37°C. HEK-Blue™ TLR cells are engineered HEK293 cells that stably co-express a human TLR gene and an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. To increase the sensitivity to their cognate agonists, HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells were further transfected with the co-receptors CD14 and MD2/CD14, respectively. Recognition of a TLR agonist by its cognate receptor triggers a signaling cascade leading to the activation of NF-κB and the production of SEAP. SEAP levels can be determined spectrophotometrically using HEK-Blue Detection or QUANTI-Blue™ Solution, both are SEAP detection media that turn purple/blue in the presence of alkaline phosphatase.

TLR2 inhibition

1. Prepare a HEK-Blue™ hTLR2 cell suspension at ~280,000 cells/ml.
2. Add 160 µl of cell suspension (~50,000 cells) per well.
3. Add 20 µl OxPAPC to obtain a final concentration 30 µg/ml in each well.
4. Incubate the plate at 37°C in a 5% CO_2 incubator for 1 h.
5. Add 20 µl of Pam3CSK4 (final concentrations 0.1 to 10 ng/ml) per well of a flat-bottom 96-well plate.
6. Incubate the plate at 37°C in a 5% CO_2 incubator for 18-24 h.
7. Monitor SEAP production using a SEAP detection assay such as QUANTI-Blue™ Solution.

TLR4 inhibition

1. Prepare a HEK-Blue™ hTLR4 cell suspension at ~150,000 cells/ml.
2. Add 160 µl of cell suspension (~25,000 cells) per well.
3. Add 20 µl OxPAPC to obtain a final concentration 30 µg/ml in each well.
4. Incubate the plate at 37°C in a 5% CO_2 incubator for 1 h.
5. Add 20 µl of LPS (final concentrations 1 ng to 100 ng/ml) per well of a flat-bottom 96-well plate.
6. Incubate the plate at 37°C in a 5% CO_2 incubator for 18-24 h.
7. Monitor SEAP production using a SEAP detection assay such as QUANTI-Blue™ Solution.

RELATED PRODUCTS

Product	Description	Cat. Code
HEK-Blue™ hTLR2 cells	TLR2 reporter cells	hkb-htlr2
HEK-Blue™ hTLR4 cells	TLR4 reporter cells	hkb-htlr4
LPS-EK Ultrapure	TLR4 agonist	tlrl-peklps
Pam3CSK4	TLR2 agonist	tlrl-pms
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs