

OxPAPC

TLR2 and TLR4 Inhibitor

Catalog # tlr1-oxp1

For research use only

Version # 10C24-MM

PRODUCT INFORMATION

Content:

- 2 x 0.5 mg OxPAPC

Resuspension of OxPAPC:

For use of total amount:

Add 500 µl of culture medium 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.

For use of partial amount:

Add 500 µl chloroform to the vial to obtain lipid concentration of 1 mg/ml and carefully vortex avoiding contact of the solvent with vial cap. Aliquot OxPAPC solution into 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. Resuspend in culture medium according as described above.

Storage and stability:

- OxPAPC is provided as a solid and shipped at room temperature. Store at -20°C. Solid product is stable 1 year at -20°C.
- Aliquots of OxPAPC resuspended in chloroform can be stored at -70°C for 3 months. Avoid repeated freeze-thaw cycles.

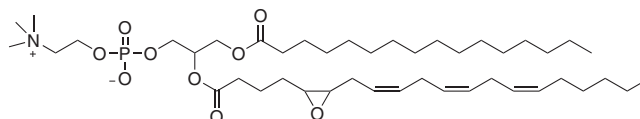
DESCRIPTION

OxPAPC is generated by the oxidation of 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphorylcholine (PAPC), which results in a mixture of oxidized phospholipids containing either fragmented or full length oxygenated sn-2 residues. OxPAPC has been shown to inhibit the signaling induced by bacterial lipopeptide and lipopolysaccharide (LPS). It acts by competing with CD14, LBP and MD2, the accessory proteins that interact with bacterial lipids, thus blocking the signaling of TLR2 and TLR4^{1,2}.

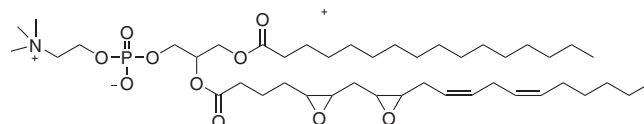
1. **Erridge C. et al., 2008.** Oxidized Phospholipid Inhibition of Toll-like Receptor (TLR) Signaling Is Restricted to TLR2 and TLR4: roles for CD14, LPS-binding protein, and MD2 as targets for specificity of inhibition. *J. Biol. Chem.*, 283: 24748-24759. 2. **von Schlieffen E. et al., 2009.** Multi-Hit Inhibition of Circulating and Cell-Associated Components of the Toll-Like Receptor 4 Pathway by Oxidized Phospholipids. *Arterioscler Thromb Vasc Biol*, 29: 356-362.

Working Concentration: 30 µg/ml

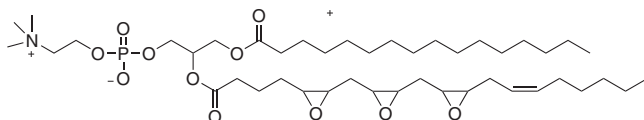
CHEMICAL PROPERTIES



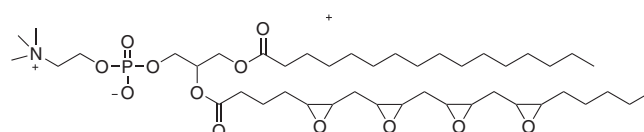
Chemical Formula: C₄₄H₈₀NO₅P
Molecular Weight: 798.08



Chemical Formula: C₄₄H₈₀NO₁₀P
Molecular Weight: 814.08



Chemical Formula: C₄₄H₈₀NO₁₁P
Molecular Weight: 830.08



Chemical Formula: C₄₄H₈₀NO₁₂P
Molecular Weight: 846.08

TECHNICAL SUPPORT

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METHODS

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 incubate 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 or NOD 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™, 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 ~250,000 cells/ml.
- 2- Add 160 µl of cell suspension (~50,000 cells) per well.
- 3- Add 20 µl OxPAPC (concentration 300 µg/ml) to obtain a final concentration 30 µg/ml in each well.
- 4- Add 20 µl of Pam3CSK4 (concentration 10 ng/ml to 1 µg/ml) to obtain final concentrations of 1 to 100 ng/ml per well of a flat-bottom 96-well plate.
- 5- Incubate the plate at 37°C in a 5% CO2 incubator for 18-24 h.
- 6- Monitor SEAP production using a SEAP detection assay such as QUANTI-Blue™.

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 (concentration 300 µg/ml) to obtain a final concentration 30 µg/ml in each well.
- 4- Add 20 µl of LPS (concentration 10 ng to 100 µg/ml) to obtain final concentrations of 1 ng to 10 µg/ml per well of a flat-bottom 96-well plate.
- 5- Incubate the plate at 37°C in a 5% CO2 incubator for 18-24 h.
- 6- Monitor SEAP production using a SEAP detection assay such as QUANTI-Blue™.

RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ hTLR2 Cells	hkb-htr2
HEK-Blue™ hTLR4 Cells	hkb-htr4
LPS-EK Ultrapure	tlrl-pekllps
Pam3CSK4	tlrl-pms
QUANTI-Blue™ (5 pouches)	rep-qb1

TECHNICAL SUPPORT

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