

TL2-C29

TLR2 signaling inhibitor - InvitroFit™

Catalog code: inh-c29

<https://www.invivogen.com/tlr2-inh-c29>

For research use only

Version 23118-MM

PRODUCT INFORMATION

Contents

- 5 mg TL2-C29 - InvitroFit™

Storage and stability

- TL2-C29 is provided as a dried powder and shipped at room temperature. Upon receipt, store at -20 °C.
- Upon resuspension, prepare aliquots and store at -20 °C. Resuspended product is stable for 2 months at -20 °C when properly stored. Avoid repeated freeze-thaw cycles.

Quality control

- Purity: ≥95% (NMR)
- The inhibitory activity of human TLR2/1 and TLR2/6 signaling has been confirmed 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

TL2-C29 is a small-molecule inhibitor of Toll-like receptor 2 (TLR2)¹. TLR2 plays an essential role in detecting a diverse range of microbial pathogen-associated molecular patterns (PAMPs) from bacteria, fungi, parasites, and viruses². TLR1 and TLR6 co-receptors are crucial for TLR2 signaling and ligand specificity. Cell surface TLR2/TLR1 and TLR2/TLR6 heterodimers bind tri- and diacylated lipoproteins, respectively^{3,4}. TLR2 signaling is initiated by ligand-induced dimerization of the essential cytoplasmic TIR (Toll/interleukin-1 receptor) domains of the TLR2 heterodimers and adapter proteins. Subsequent signaling cascades lead to a pro-inflammatory response.

TL2-C29 binds a pocket in the BB loop within the TLR2 TIR domain, thereby inhibiting TLR2 interaction with the MyD88 adapter molecule and downstream MAPK and NF-κB activation¹. In human TLR2 signaling, TL2-C29 blocks both TLR2/1 and TLR2/6 pathways. In murine TLR2 signaling, TL2-C29 preferentially blocks the TLR2/1 pathway¹.

1. Mistry, P. et al., 2015. Inhibition of TLR2 signaling by small molecule inhibitors targeting a pocket within the TLR2 TIR domain. PNAS. 112(17):5455-5460. **2. Oliveira-Nascimento L. et al., 2012.** The role of TLR2 in infection and immunity. Front Immunol. 3(79): doi:10.3389/fimmu.2012.00079. **3. Takeuchi O. et al., 2001.** Discrimination of bacterial lipoproteins by Toll-like receptor 6. Int Immunol. 13: 933-940. **4. Takeuchi O. et al., 2002.** Cutting edge: role of Toll-like receptor 1 in mediating immune response to microbial lipoproteins. J Immunol. 169: 10-14.

CHEMICAL PROPERTIES

Synonyms: C29; TLR2-IN-C29; 3-[[[(2-hydroxy-3-methoxyphenyl)methylene]amino]-2-methyl-benzoic acid

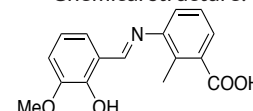
CAS number: 363600-92-4

Formula: C₁₆H₁₅NO₄

Molecular weight: 285.3 g/mol

Solubility: 50 mM (14.3 mg/ml) in DMSO

Chemical structure:



METHODS

Preparation of stock solution (50 mM)

1. Add 350 µl DMSO to 5 mg TL2-C29.
2. Vortex until completely resuspended.
3. Prepare aliquots of TL2-C29 and store at -20 °C.
4. Once TL2-C29 is resuspended, further dilutions can be prepared using sterile saline buffers (e.g. PBS). *Note: TL2-C29 is not soluble in water.*

Working concentration range in cellular assays:

- 25 µM - 200 µM for hTLR2/1 signaling inhibition
- 10 µM - 200 µM for hTLR2/6 signaling inhibition

PROTOCOL

Below is a protocol using InvivoGen's **HEK-Blue™ hTLR2 cells** for studying the specific inhibition of human TLR2 signaling by TL2-C29. These cells express an inducible secreted embryonic alkaline phosphatase (SEAP) reporter to readily measure the activation of the NF-κB pathway. Changes in SEAP expression due to inhibition of TLR2 signaling can be assessed using **QUANTI-Blue™ Solution**, a SEAP detection reagent.

1. Add 20 µl TL2-C29 (10x conc) per well of a flat bottom 96-well plate.
2. Prepare a suspension of **HEK-Blue™ hTLR2 cells** (~310,000 cells per ml) in culture medium.
3. Add 160 µl of the cell suspension (~50,000 cells) to each well.
4. Incubate the plate at 37 °C in a CO₂ incubator for 3 hours.
5. Add 20 µl (10X conc) of an inducer of TLR2 signaling (e.g. **Pam3CSK4** or **FSL-1**) and incubate the plate at 37 °C in a CO₂ incubator for 24 hours.
6. Prepare **QUANTI-Blue™ Solution** and carry out the measurements following the instructions on the data sheet.

RELATED PRODUCTS

Product	Cat. Code
HEK-Blue™ hTLR2 cells	hkb-htlr2
QUANTI-Blue™ Solution	rep-qbs
Pam3CSK4	tlrl-pms
FSL-1	tlrl-fsl

TECHNICAL SUPPORT

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