

Isoliquiritigenin

NLRP3 inflammasome inhibitor

Catalog code: inh-ilg

<https://www.invivogen.com/isoliquiritigenin>

For research use only

Version 2014-MM

PRODUCT INFORMATION

Contents:

- 10 mg Isoliquiritigenin provided lyophilized

Storage and stability:

- Product is shipped at room temperature. Upon receipt, store at -20°C.
- Upon resuspension, prepare aliquots and store at -20°C. Resuspended product is stable for at least 6 months when properly stored. Avoid repeated freeze-thaw cycles.

Quality control:

- Purity ≥98% (UHPLC)
- The inhibitory activity has been validated using in-house 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

Isoliquiritigenin is a broad-spectrum inhibitor with multiple effects, including anti-inflammatory, anti-oxidant, and anti-tumor activities¹. Its targets include NF-κB and the NLRP3 inflammasome. The NLRP3 inflammasome is a multi-protein complex comprising the NLR protein NLRP3, the adapter ASC and pro-caspase-1. It is activated by a two-step process; a first signal ('priming') is provided mainly by bacterial components or endogenous cytokines involves NF-κB induction, while the second signal provided by a wide array of stimuli including microbial toxins, endogenous molecules or crystalline substances and leads to inflammasome assembly and activation. This triggers inflammasome multimerization and caspase-1 activation with the subsequent cleavage of interleukin-1β (IL-1β)/IL-18 and the pore forming protein Gasdermin D (GSDMD) into their active forms. Additionally, the activation of the inflammasome also leads to alarmin secretion and pyroptosis, a form of immunogenic cell death.

Specifically, isoliquiritigenin inhibits TNF-α-induced NF-κB activation and translocation to the nucleus by blocking IκB kinase activity, suggesting that it can block inflammasome priming². Another report suggests it may exhibit inhibitory action on NLRP3-induced ASC oligomerization³. In addition, it has been reported that isoliquiritigenin is more potent than other inflammasome inhibitors such as parthenolide and glybenclamide³. Of note, isoliquiritigenin does not inhibit the AIM2 inflammasome³.

1. Jung SK, et al., 2014. Isoliquiritigenin induces apoptosis and inhibits xenograft tumor growth of human lung cancer cells by targeting both wildtype and L858R/T790M mutant EGFR. *J. Biol. Chem.* 289(52):35839-48. 2. Kumar S, et al., 2007. Isoliquiritigenin inhibits IκB kinase activity and ROS generation to block TNF-α induced expression of cell adhesion molecules on human endothelial cells. *Biochem. Pharmacol.* 73(10): 1602-12. 3. Honda H, et al., 2014. Isoliquiritigenin is a potent inhibitor of NLRP3 inflammasome activation and diet-induced adipose tissue inflammation. *J. Leukoc. Biol.* 96(6):1087-100.

CHEMICAL PROPERTIES

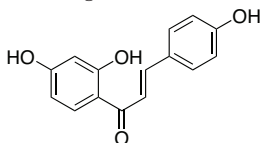
Solubility: 10 mg/ml in DMSO and 25 mg/ml in ethanol

CAS number: 961-29-5

Formula: C₁₅H₁₂O₄

Molecular weight: 256 g/mol

Structure:



METHODS

Preparation of 1 mg/ml (3.9 mM) stock solution

1. Add 1 ml of DMSO to 10 mg isoliquiritigenin. Mix by vortexing.
2. Prepare a 1:10 dilution with DMSO to obtain a 1 mg/ml solution
3. Prepare further dilutions using sterile endotoxin-free water.

Working concentration: 1 to 50 µg/ml for cell culture assays

In vitro inhibition of the NLRP3 inflammasome:

The following protocol describes the monitoring of NLRP3 inflammasome inhibition in human THP1-Null2 cells by assessing the inhibition of IL-1β production.

1. Prepare a THP1-Null2 cell suspension and add 3 x 10⁵ cells per well in a 96-well plate.
2. Prime cells by adding 1 µg/ml LPS-EK for 3 hours at 37°C in 5% CO₂.
3. Gently remove medium and add 180 µl of fresh test medium.
4. Stimulate cells by adding IL-1β inducers, such as MSU crystals (100-200 mg/ml) in the presence or absence of Isoliquiritigenin (1-50 µg/ml).
5. Incubate from 6 hours to overnight at 37°C in 5% CO₂.
6. Determine caspase-1 inhibition by detecting mature IL-1β with InvivoGen's HEK-Blue™ IL-1β cells, which are specifically engineered to detect bioactive IL-1β.

PROTOCOLS

For reference only; as described in the indicated publications.

Cell Culture Assay²

Cells: RAW 264.7 cells

Working concentration: 30 µM (7.7 µg/ml)

Pre-incubation: 30 minutes

Method: NF-κB activation was monitored by measuring GFP expression from a reporter construct using flow cytometry.

Animal Study²

Animal model: BALB/c mice

Dose: 50 mg/kg

Administration: Orally

RELATED PRODUCTS

Product	Description	Cat. Code
LPS-EK	LPS from <i>E. coli</i> K12	tlrl-eklps
MSU Crystals	Inflammasome inducer	tlrl-msu
Poly(dA:dT)	Inflammasome inducer	tlrl-patn
THP1-Null2 Cells	Human monocytes	thp-nullz
HEK-Blue™ IL-1β cells	IL-1β reporter cells	hkb-il1b
VX-765	Caspase -1 and -4 inhibitor	inh-vx765i-1

TECHNICAL SUPPORT

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