

G140

Human cGAS inhibitor - InvitroFit™

Catalog Code: inh-g140, inh-g140-5

<https://www.invivogen.com/g140>

For research use only

Version 24A02-MM

PRODUCT INFORMATION

Contents G140 is available in two quantities:

- **inh-g140:** 2 mg G140 - InvitroFit™
- **inh-g140-5:** 5 x 2 mg G140 - InvitroFit™

Storage and stability

- G140 is provided as a dried powder and shipped at room temperature. Upon receipt, store product at -20°C.
- Upon resuspension of G140 in DMSO prepare aliquots and store at -20°C. Resuspended product is stable for up to 6 months when properly stored at -20°C.
- Avoid repeated freeze-thaw cycles.

Quality control

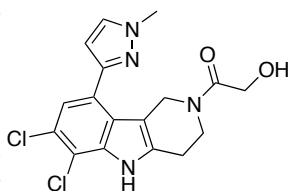
- Purity: ≥95% (UHPLC)
- Inhibition of cGAS by G140 has been confirmed using cellular assays.
- Absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and TLR4 cellular assays.

PRODUCT DESCRIPTION

G140 is small molecule inhibitor of the double-stranded DNA (dsDNA) sensor, cGAS (cyclic GMP-AMP synthase; cGAMP synthase). G140 was developed from a molecular scaffold (i.e. G001) identified by *in vitro* high-throughput screening (HTS) targeted towards recombinant human cGAS. The inclusion of a methyl pyrazole group in G140 significantly improves its potency and inhibitory activity against human (h)cGAS¹.

cGAS is the primary sensor of cytosolic dsDNA that activates the interferon (IFN) pathway, such as during bacterial or viral infection. Furthermore, cellular stress and genetic factors (e.g. autoimmune diseases) can also lead to aberrant cellular dsDNA and thus, can activate cGAS². In some autoinflammatory diseases, such as systemic lupus erythematosus (SLE) cGAS can be constitutively activated due to mis-localized self-DNA, and ultimately, cause high type I IFN production and interferonopathies³.

G140 has been shown to specifically inhibit hcGAS, with no off-target effects on a variety of different sensors such as TLRs, STING or RNA sensors¹. Additionally, G140 has a dose-dependent inhibitory activity in both monocytes (i.e. THP1 cells) and primary human macrophages¹. cGAS-mediated STING activation has also been implicated in NF-κB activation³. G140 has been shown to also exhibit inhibitory activity on cGAS-mediated activation of NF-κB¹.



CHEMICAL PROPERTIES

Synonym: 1-(6,7-Dichloro-9-(1-methyl-1H-pyrazol-3-yl)-1,3,4,5-tetrahydro-2H-pyrido[4,3-b]indol-2-yl)-2-hydroxyethan-1-one

Formula: C₁₇H₁₆Cl₂N₄O₂

Molecular weight: 379.24 g/mol

Solubility: 5 mg/mL DMSO

METHODS

Preparation of 5 mg/ml (13.18 mM) stock solution of G140

1. Resuspend 2 mg of G140 in 400 µl of DMSO. Mix by vortexing.
2. Use immediately or store aliquots at -20°C.

Note: Subsequent dilutions into the working concentration range can be performed with cell culture medium.

Working concentration range: 1 - 20 µM (for cell culture assays)

Specific inhibition of human cGAS by G140 in cellular assays

Below is a protocol for monitoring cGAS inhibition by G140 using InvivoGen's THP1-Dual™ cells. These cells allow the simultaneous study of the NF-κB pathway, by monitoring the activity of SEAP, and the IRF (interferon regulatory factor) pathway, by assessing the activity of the secreted Lucia luciferase. Additionally, to achieve cGAS stimulation, dsDNA must be delivered to the cytoplasm by using a transfection agent, such as LyoVec™.

Note: For more information, visit <https://www.invivogen.com/thp1-dual>

1. Add 20 µl of G140 (10X concentration) per well of a round bottom 96-well plate.
2. Prepare a suspension of THP1-Dual™ cells (~625,000 cells per ml).
3. Add 160 µl of cell suspension (~100,000 cells) per well.
4. Incubate at 37°C for 3 hours.
5. Add 20 µl of a test sample
6. To another well, add 20 µl of a cGAS ligand such as double-stranded DNA (e.g. G3-YSD) complexed to a transfection reagent (e.g. LyoVec™) at 1 µg/ml (final concentration)
7. Incubate the plate at 37°C in a 5% CO₂ incubator for 18-24 hours.
8. Prepare QUANTI-Luc™ 4 Lucia/Gaussi (IRF assessment) and/or QUANTI-Blue Solution (NF-κB assessment) and carry out the measurements following the instructions on the data sheet.

RELATED PRODUCTS

Product	Description	Cat. Code
G3-YSD	cGAS agonist	tlrl-ydna
THP1-Dual™ cells	Human reporter cells	thpd-nfis
THP1-Dual™ KO-cGAS cells	Human KO reporter cells	thpd-kocgas

1. Lama, L. *et al.* 2019. Development of human cGAS-specific small-molecule inhibitors for repression of dsDNA-triggered interferon expression. *Nat Commun* 10, 2261. 2. Gao, D. *et al.* 2015. Activation of cyclic GMP-AMP synthase by self-DNA causes autoimmune diseases. *PNAS* 112, E5699-5705. 3. Abe, T. *et al.* 2014. Cytosolic-DNA-mediated, STING-dependent proinflammatory gene induction necessitates canonical NF-κB activation through TBK1. *J Virol* 88, 5328-5341.

TECHNICAL SUPPORT

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