H-151

STING inhibitor

Catalog code: inh-h151

https://www.invivogen.com/h151

For research use only

Version 19G19-MM

PRODUCT INFORMATION

Contents

• 10 mg H-151

Storage and stability

- H-151 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 3 months when properly stored. Avoid repeated freeze-thaw cycles.

Quality control

- Purity ≥ 95% (UHPLC)
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.
- The inhibitory activity has been validated using cellular assays.

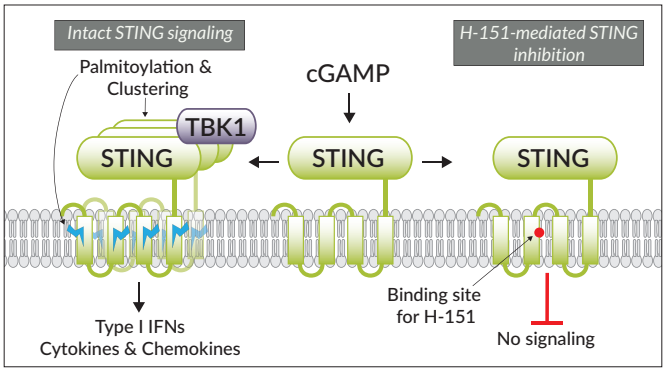
BACKGROUND

STING (stimulator of interferon genes) has become a focal point in immunology research and drug discovery[1, 2]. In a healthy individual, STING functions as a signaling hub, orchestrating immune responses to pathogenic, tumoral, or self-DNA detected in the cytoplasm[2]. Upon activation, STING induces type I interferon (IFN) production through TANK-binding-kinase-I (TBK1)-mediated IFN regulatory factor (IRF3) signaling[2]. STING activation also leads to NF-κB-dependent inflammatory cytokine production[2]. In some autoimmune diseases such as STING-associated vasculopathy with onset in infancy (SAVI), STING is constitutively activated resulting in high IFN production[3, 4]. The discovery of a mechanism to pharmacologically inhibit STING should lead to new treatments for such diseases.

PRODUCT DESCRIPTION

H-151 is a potent, irreversible and selective small molecule inhibitor of STING[1]. This synthetic indole-derivative exerts its inhibitory action by covalently binding to STING at the transmembrane cysteine residue at position 91. H-151 blocks STING palmitoylation and clustering, two essential steps for STING signaling. Of note, H-151 potently inhibits both human and murine STING, *in vitro* and *in vivo* mouse models. Indeed, in models of autoinflammatory disease, H-151 blocks STING-induced expression of pro-inflammatory cytokines and reduces inflammation[1]. Notably, H-151 is effective against all the STING variants tested, including constitutively active disease-associated mutants such as S154 (N154S) and M155 (V155M; see validation data sheet available on our website).

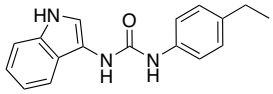
1. Haag S.M. *et al.*, 2018. Targeting STING with covalent small-molecule inhibitors. Nature 559:269-73. **2. Ishikawa H. & Barber G.N. 2008.** STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. Nature 455:674-8. **3. Liu Y. *et al.*, 2014.** Activated STING in a vascular and pulmonary syndrome. N Engl J Med. 371:507-18. **4. Jeremiah N. *et al.*, 2013.** Inherited STING-activating mutation underlies a familial inflammatory syndrome with lupus-like manifestations. J Clin Invest. 124:5516-20.



Mechanism of action of H-151-mediated STING inhibition.

CHEMICAL PROPERTIES

CAS number: 941987-60-6
Synonym: N-(4-Ethylphenyl)-N'-1H-indol-3-yl-urea
Solubility: 20 mg/ml (71.60 mM) in DMSO
Formula: $C_{17}H_{17}N_3O$
Molecular weight: 279.34 g/mol
Structure:



METHODS

Preparation of 10 mg/ml (35.8 mM) stock solution

1. Add 1 ml of DMSO to 10 mg of H-151. Mix by vortexing.
2. Use immediately or store aliquots at -20 °C.
3. Prepare a 1:10 dilution with DMSO to obtain a 1 mg/ml solution.
4. Further dilutions of the 1 mg/ml solution can be prepared using culture medium, such as RPMI or DMEM, containing 10 % fetal calf serum.

Note: *Dilutions in water or PBS may cause the product to precipitate.*

Working concentration range: 4 ng/ml (15 nM) to 4 µg/ml (15 µM) for cellular assays

STING inhibition assay

On the next page is a protocol to study STING inhibition in THP1-Dual™ cells. These cells derive from the human monocytic cell line THP-1, by stable integration of two inducible reporters allowing the simultaneous study of the IRF pathway, by assessing the activity of the secreted luciferase Lucia, and the NF-κB pathway, by monitoring the activity of SEAP. These cells have been shown to express STING and respond to STING agonists.
For more information, visit https://www.invivogen.com/thp1-dual.

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Protocol for STING inhibition in THP1-Dual™ cells

1. Add 20 µl of H-151 (final concentration 4 ng/ml to 4 µg/ml) per well of a flat-bottom 96-well plate.
2. Add 160 µl of cell suspension (~100,000 cells) per well.
3. Incubate for 2 hours at 37 °C in a 5% CO_2 incubator.
4. Add 20 µl of a test sample or a STING agonist, such as 2'3'-cGAMP (final concentration 20 µg/ml) per well.
5. Incubate the plate for 18-24 hours at 37 °C in a 5% CO_2 incubator.
6. Monitor IRF and NF-κB activation by measuring the levels of Lucia luciferase and SEAP using QUANTI-Luc™ and QUANTI-Blue™ Solution, respectively.

STING inhibition assays can also be performed in other cells lines from InvivoGen's STING reporter cell collection. InvivoGen has developed stable reporter cells where the wild-type STING gene has been replaced by a STING variant using knock-in technology (KI-STING). These cells feature IRF-inducible Lucia luciferase and an NF-κB SEAP (secreted embryonic alkaline phosphatase) secreted reporter proteins as convenient read-outs.
For more information, visit https://www.invivogen.com/sting-reporter-cells.

RELATED PRODUCTS

Product	Cat. Code
STING Agonists	
2'3'-cGAMP	tlrl-nacga23
3'3'-cGAMP Fluorinated	tlrl-nacgaf
2'3'-c-di-AM(PS)2 (Rp,Rp)	tlrl-nacda2r
Detection Reagents	
QUANTI-Blue™ Solution	rep-qbs
QUANTI-Luc™	rep-qlc1
Mouse Macrophage Reporter Cells Lines	
RAW-Lucia™ ISG Cells	rawl-isg
RAW-Lucia™ ISG-KO-STING cells	rawl-kostg
Human Monocytic Reporter Cells Lines	
THP1-Dual™ Cells	thpd-nfis
THP1-Dual™ KO-STING Cells	thpd-kostg
THP1-Dual™ KI-hSTING-A162 Cells	thpd-a162
THP1-Dual™ KI-hSTING-H232 Cells	thpd-h232
THP1-Dual™ KI-hSTING-M155 Cells	thpd-m155
THP1-Dual™ KI-hSTING-R232 Cells	thpd-r232
THP1-Dual™ KI-hSTING-S154 Cells	thpd-s154