

# ODN TTAGGG (A151)

## Suppressive oligonucleotide TLR9, AIM2 and cGAS antagonist

Catalog code: tlr1-ttag151, tlr1-ttag151-1

<http://www.invivogen.com/odnttaggg>

For research use only

Version 18D10-MM

### PRODUCT INFORMATION

#### Contents

• ODN TTAGGG (A151) is provided lyophilized and available in two quantities:

- 200 µg (25.2 nmol): tlr1-ttag151
- 1 mg (126 nmol): tlr1-ttag151-1

*Note:* ODN TTAGGG (A151) is sterile filtered prior to lyophilization.

- 1.5 ml endotoxin-free water

#### ODN TTAGGG (A151) sequence

5'- tt agg gtt agg gtt agg gtt agg g -3' (24 mer)

*Note:* Bases are phosphorothioate-linked (nuclease resistant).

**Molecular weight:** 7944 g/mol

#### Storage and stability

- ODN TTAGGG (A151) is shipped at room temperature. Upon receipt, store at -20°C.
- Upon resuspension, store aliquots at -20°C. Resuspended product is stable for 6 months at -20°C when properly stored. Avoid repeated freeze-thaw cycles.

#### Quality control

- Biological activity has been tested using HEK-Blue™ TLR9 cells.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

### DESCRIPTION

ODN TTAGGG (A151), also known simply as A151, is a synthetic oligonucleotide (ODN) containing 4 repeats of the immunosuppressive TTAGGG motif commonly found in mammalian telomeric DNA<sup>1</sup>. Initially, this ODN was identified as a TLR9 antagonist that inhibits immune activation by CpG-containing ODNs<sup>2</sup>. Of note, its inhibitory activity is stronger towards human TLR9 compared to its murine counterpart. The cytosolic DNA sensors (CDSs) AIM2 and IFI16 were subsequently identified as additional targets for this inhibitor<sup>3, 4</sup>. By binding to these CDSs, it prevents AIM2 inflammasome activation<sup>3</sup>. Interestingly, ODN TTAGGG (A151) was reported as a cGAS inhibitor, acting through competition with DNA<sup>1</sup>. Overall, these findings show that ODN TTAGGG (A151) is a multiple pattern recognition receptors (PRR) suppressor that can prove useful for immunomodulatory studies.

1. **Steinhagen F. et al., 2017.** Suppressive oligodeoxynucleotides containing TTAGGG motifs inhibit cGAS activation in human monocytes. *Eur J Immunol*. DOI: 10.1002/eji.201747338. 2. **Gursel I. et al., 2003.** Repetitive elements in mammalian telomeres suppress bacterial DNA-induced immune activation. *J Immunol*. 171(3):1393-400. 3. **Eichholz K. et al., 2016.** Immune-Complexed Adenovirus Induce AIM2-Mediated Pyroptosis in Human Dendritic Cells. *PLoS Pathog*. 12(9):e1005871. 4. **Kaminski J. et al., 2013.** Synthetic oligodeoxynucleotides containing suppressive TTAGGG motifs inhibit AIM2 inflammasome activation. *J Immunol*. 191(7):3876-83.

### METHODS

#### Preparation of ODN TTAGGG (A151) solution (500 µM)

- Resuspend product with endotoxin-free water (provided).
  - Add 50 µl to 200 µg vial of ODN TTAGGG (A151)
  - Add 250 µl to 1 mg vial of ODN TTAGGG (A151)
- Vortex until completely dissolved. Prepare aliquots and store at -20°C.

**Working concentration:** 100 nM-10 µM

#### Inhibition of CpG-ODN-mediated TLR9 activity

Inhibition of TLR9 activity is typically achieved with a 1:1-10:1 ratio of inhibitory ODN:stimulatory ODN. HEK-Blue™ TLR9 cell can be used to study the inhibitory activity of ODN TTAGGG (A151) against TLR9. These cells stably overexpress the TLR9 gene and an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. For more information, <http://www.invivogen.com/hek-blue-tlr9>.

1. Dispense 20 µl of stimulatory ODN in each well of a 96-well plate.

*Note:* We recommend to test several concentrations of the stimulatory ODN and inhibitory ODN, 3 or 10-fold apart.

2. Add 20 µl of ODN TTAGGG (A151) per well (100 nM-10 µM final concentration).
3. Distribute 160 µl of cell suspension (4-8 x10<sup>4</sup> cells) to each well.
4. Incubate for 6-24 h at 37°C, 5% CO<sub>2</sub>.
5. Determine inhibition of TLR9 activity by assessing SEAP expression using QUANTI-Blue™ Solution, a SEAP detection medium.

#### Inhibition of AIM2 activity

THP1-HMGB1-Lucia™ cells can be used to study the inhibitory activity of ODN TTAGGG (A151) against AIM2. These cells derive from THP-1 human monocytes and stably express the fusion protein HMGB1::Lucia. They are typically primed with LPS before treatment with inflammasome inducers. Inflammasome activation leads to pyroptosis-mediated release of HMGB1::Lucia and IL-1β in the extracellular milieu. For more information, <http://www.invivogen.com/thp1-hmgb1-lucia>.

1. Dispense 20 µl of LPS-EK (1 µg/ml final concentration) per well of a 96-well plate.
2. Distribute 180 µl of cell suspension (2 x10<sup>5</sup> cells) to each well.
3. Incubate at 37°C in 5% CO<sub>2</sub> for 3 h.
4. Gently remove medium and add 160 µl of fresh test medium.
5. Add 20 µl of ODN TTAGGG (A151) per well (100 nM-10 µM final concentration).
6. Add 20 µl of an AIM2 inflammasome inducer such as poly(dA:dT) delivered to the cytosol (500 ng/ml final concentration).
7. Incubate for 16-24 h at 37°C, 5% CO<sub>2</sub>.
8. Determine inhibition of AIM2 activity by measuring HMGB1::Lucia in the supernatant using QUANTI-Luc™, a luciferase detection medium. Levels of IL-1β can be measured by ELISA or using InvivoGen's HEK-Blue™ IL-1β cellular assay.

### TECHNICAL SUPPORT

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### Inhibition of cytosolic DNA sensors (CDSs) activity

Inhibition of CDS activity with ODN TTAGGG (A151) can be studied in a variety of cells, including THP1-Dual™ cells which express multiple CDSs and an interferon regulatory factor (IRF)-inducible Lucia luciferase reporter gene.

For more information, <http://www.invivogen.com/thp1-dual>.

1. Dispense 20 µl of ODN TTAGGG (A151) in each well of a 96-well plate (100 nM-10 µM final concentration).
2. Add 160 µl of THP1-Dual™ cell suspension (1 x 10<sup>5</sup> cells) to each well.
3. Incubate for 6 h at 37°C, 5% CO<sub>2</sub>.
4. Add 20 µl of CDS agonists such as dsDNA delivered to the cytosol (1 µg/ml final concentration) per well.
5. Incubate for 16-48 hours at 37°C, 5% CO<sub>2</sub>.
6. Determine inhibition of CDS activity by measuring the levels of Lucia luciferase using QUANTI-Luc™, a secreted luciferase detection reagent.

### RELATED PRODUCTS

Product	Description	Cat. Code
HEK-Blue™ IL-1β Cells	IL-1β Sensor cells	hkb-il1b
HEK-Blue™ hTLR9 Cells	TLR9 Reporter cells	hkb-hltr9
LyoVec™	Transfection reagent	lyec-12
ODN 2006	Stimulatory CpG ODN	tlrl-2006
ODN TTAGGG Control	Negative control	tlrl-ttagc
Poly(dA:dT)/LyoVec™	AIM2 inflammasome inducer	tlrl-patc
QUANTI-Blue™ Solution	SEAP detection medium	rep-qbs1
QUANTI-Luc™	Luciferase detection medium	rep-qlc1
THP1-Dual™ cells	Reporter monocytes	thpd-nfis
THP1-HMGB1-Lucia™ Cells	Pyroptosis reporter monocytes	thp-gb1lc

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