

# ODN TTAGGG Control

Negative control for ODN TTAGGG (A151)

Catalog code: tlrl-ttagc-1

<https://www.invivogen.com/odnttaggg-control>

For research use only

Version 21L20-MM

## PRODUCT INFORMATION

### Contents

- 1 mg (206 nmol) ODN TTAGGG Control provided lyophilized
- Note: ODN TTAGGG Control is sterile filtered prior to lyophilization.*
- 1.5 ml endotoxin-free water

### ODN TTAGGG Control sequence

5'-gctagatgttagcgt-3' (15 mer)

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

**Molecular weight:** 4848 g/mol

### Storage and stability

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

### Quality control

- The absence of inhibitory activity has been confirmed 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 Control is a negative control for the suppressive ODN TTAGGG (A151). ODN TTAGGG (A151), also known 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>5</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. Krieg A. et al., 1998.** Sequence motifs in adenoviral DNA block immune activation by stimulatory CpG motifs. *PNAS* 95(21):12631-6. **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 solution (500 µM)

- Add 415 µl of endotoxin-free water (provided) to 1 mg vial of ODN TTAGGG Control
- Vortex until completely dissolved. Prepare aliquots and store at -20°C.

### Inhibition of CpG ODN stimulation

ODN TTAGGG Control can be used as a control ODN to study the inhibitory effect of ODN TTAGGG. Inhibition of CpG ODN stimulation is typically achieved with a 1-10:1 ratio of inhibitory ODN:stimulatory ODN. The inhibitory activity of ODN TTAGGG on TLR9 can be assessed using HEK-Blue™ TLR9 cells. These cells stably overexpress the TLR9 gene and an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene.

For more information, visit: <https://www.invivogen.com/hek-blue-tlr9>.

Below is a protocol to study TLR9 inhibition using HEK-Blue™ TLR9 cells in a 96-well plate.

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

1. Dispense 20 µl of stimulatory ODN per well in a column, at concentrations ranging from 0 to 10 µM.
2. Add 20 µl of inhibitory or control ODN per well in a row, at concentrations ranging from 0 to 10 µM.
3. Prepare cell suspension of HEK-Blue™ TLR9 cells according to the data sheet.
4. Add HEK-Blue™ TLR9 cells (4-8 x10<sup>4</sup>) to each well.
5. Incubate for 6-24 h at 37°C, 5% CO<sub>2</sub>.
6. Determine inhibition of TLR9 stimulation by assessing cytokine expression using ELISA, or SEAP expression using QUANTI-Blue™ Solution, a SEAP detection medium.

## RELATED PRODUCTS

Product	Description	Cat. Code
HEK-Blue™ hTLR9 cells	Human TLR9 reporter cells	hkb-htlr9
HEK-Blue™ mTLR9 cells	Murine TLR9 reporter cells	hkb-mtlr9
ODN 2216	Stimulatory ODN	tlrl-2216
ODN TTAGGG	Suppressive ODN	tlrl-ttag151
QUANTI-Blue™ Solution	SEAP detection medium	rep-qbs

## TECHNICAL SUPPORT

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