ODN TTAGGG (A151)
Suppressive oligonucleotide
TLR9, AIM2 and cGAS antagonist
Catalog code: tlrl-ttag151, tlrl-ttag151-1
http://www.invivogen.com/odnttaggg
For research use only
Version 18D09-MM

PRODUCT INFORMATION

Contents
• ODN TTAGGG (A151) is provided lyophilized and available in two quantities:
  - 200 µg (25.2 nmol): tlrl-ttag151
  - 1 mg (126 nmol): tlrl-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. Initially, this ODN was identified as a TLR9 antagonist that inhibits immune activation by CpG-containing ODNs. 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. By binding to these CDSs, it prevents AIM2 inflammasome activation. Recently, ODN TTAGGG (A151) was reported as a cGAS inhibitor, acting through TLR4 cells.

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)

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-kB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene.


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) (100 nM-10 µM final concentration).
3. Distribute 160 µl of cell suspension (4-8 x10^4 cells) to each well.
4. Incubate for 6-24 h at 37°C, 5% CO_2.
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 x10^5 cells) to each well.
3. Incubate at 37°C in 5% CO_2 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).

Level of IL-1β can be measured by ELISA or using InvivoGen’s HEK-Blue™ IL-1β ELISA kit.

Note: 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 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) (100 nM-10 µM final concentration).
3. Distribute 160 µl of cell suspension (4-8 x10^4 cells) to each well.
4. Incubate for 6-24 h at 37°C, 5% CO_2.
5. Determine inhibition of TLR9 activity by assessing SEAP expression using QUANTI-Blue™ Solution, a SEAP detection medium.

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 x10^5 cells) to each well.
3. Incubate at 37°C in 5% CO_2 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).

Level of IL-1β can be measured by ELISA or using InvivoGen’s HEK-Blue™ IL-1β ELISA kit.

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 x10^5 cells) to each well.
3. Incubate at 37°C in 5% CO_2 for 3 h.
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.


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 10⁵ cells) to each well.
3. Incubate for 6 h at 37 °C, 5% CO₂.
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% CO₂.
6. Determine inhibition of CDS activity by measuring the levels of Lucia luciferase using QUANTI-Luc™, a secreted luciferase detection reagent.

RELATED PRODUCTS

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Cat. Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEK-Blue™ IL-1β Cells</td>
<td>IL-1β Sensor cells</td>
<td>hkb-il1b</td>
</tr>
<tr>
<td>HEK-Blue™ hTLR9 Cells</td>
<td>TLR9 Reporter cells</td>
<td>hkb-htlr9</td>
</tr>
<tr>
<td>LyoVec™</td>
<td>Transfection reagent</td>
<td>lyec-12</td>
</tr>
<tr>
<td>ODN 2006</td>
<td>Stimulatory CpG ODN</td>
<td>ttrl-2006</td>
</tr>
<tr>
<td>ODN TTAGGG Control</td>
<td>Negative control</td>
<td>ttrl-ttagc</td>
</tr>
<tr>
<td>Poly(dA:dT)/LyoVec™</td>
<td>AIM2 inflammasome inducer</td>
<td>ttrl-pate</td>
</tr>
<tr>
<td>QUANTI-Blue™ Solution</td>
<td>SEAP detection medium</td>
<td>rep-qbs1</td>
</tr>
<tr>
<td>QUANTI-Luc™</td>
<td>Luciferase detection medium</td>
<td>rep-qlc1</td>
</tr>
<tr>
<td>THP1-Dual™ cells</td>
<td>Reporter monocytes</td>
<td>thpd-nfis</td>
</tr>
<tr>
<td>THP1-HMGB1-Lucia™ Cells</td>
<td>Pyroptosis reporter monocytes</td>
<td>thp-gb1le</td>
</tr>
</tbody>
</table>

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3622-3480
E-mail: info@invivogen.com