



## PRODUCT DATA SHEET

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### iODN (inhibitory ODN) 2088 Endotoxin-free (sterile)

**Cat. No.:** IAX-200-050

**Lot. No.:**

|                       |   |
|-----------------------|---|
| <b>Sequence</b>       | 5'-tcctggcggggaagt-3' (lower case letters: phosphorothioate linkage: nuclease resistant)  |
| <b>Synonyms</b>       | Inhibitory ODN (iODN); Class I  |
| <b>MW</b>             | 4,887 g/mol   |
| <b>Formulation</b>    | Lyophilised. Sterile. 100µg size includes 1.5ml ddWater Endotoxin-free (sterile) (Cat. No.: IAX-900-002-LD15). 1mg size includes 10ml ddWater Endotoxin-free (sterile) (Cat. No.: IAX-900-002-L010).  |
| <b>Endotoxin-free</b> | Bacterial Endotoxin Test (kinetic turbidimetric LAL method) according to Ph. Eur. 9.<br>Passed according to specification:<br>Endotoxin-free: <0.002 EU/µg.   |
| <b>Sterility</b>      | Filter method: according to Ph. Eur. 9.<br>Passed according to specification:<br>• No growth in Thioglycolate medium at 30-35°C after 14 days.<br>• No growth in Soybean Casein Digest Broth (TSB) at 20-25°C after 14 days.                          |
| <b>Handling</b>       | Keep sterile. Reconstitution: Dissolve total vial content in sterile endotoxin-free water or PBS. Add 50% of solvent and let dissolve for 10mins. Add remaining 50% of the solvent and mix thoroughly. Moderate warming may aid dissolving.           |
| <b>Activity</b>       | Potent sequence of an inhibitory ODN for <i>in vivo</i> use in rodents (50-150µg per injection): prototype class I, may also affect TLR7 and TLR8 signalling.<br>Negative control: CTRL2-ODN (Control for iODN and CpG-ODNs) (Cat. No.: IAX-200-208). |
| <b>Shipping</b>       | Ambient   |
| <b>Storage</b>        | 2-8°C. After reconstitution in water prepare aliquots, store between -15°C and -25°C (shelf-life: 6 months). Avoid freeze/thaw cycles. After thawing stable for one day at 2-8°C, and do not freeze again.  |
| <b>Stability</b>      | 2 years after receipt (unopened and as supplied)  |
| <b>MSDS</b>           | Available on request  |

**Document No.:** IAX-200-050 | **Version:** 1.5 | **Issue Date:** 30/01/2023

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**MATERIAL SAFETY DATA:** This material should be considered hazardous until information to the contrary becomes available. Do not ingest, swallow, inhale or get into the blood stream. Do not get in eyes, on skin, or clothing. Wash thoroughly after handling. This information contains some, but not all, of the information required for the safe and proper use of this material. Access to this material must be restricted to personnel, who is appropriately experienced, qualified, competent and properly trained to use it. Material Safety Data Sheet is available upon request.

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#### Product Specific References

[1] *Respiratory Syncytial Virus-Infected Mesenchymal Stem Cells Regulate Immunity via Interferon Beta and Indoleamine-2,3-Dioxygenase*. Cheun MB, et al. PLOS ONE (2016); 11:e0163709

- In recent years several groups have studied the sequence requirements, specificity, signalling pathways and kinetics of the TLR (Toll-like receptor) 9 suppression by inhibitory oligonucleotide motifs, which led to a class of novel **inhibitory oligonucleotide (iODNs)**, that is independent of the previously thought species preference. Subsequently it has been discovered that telomeric DNA repeats (TTAGGG)<sub>n</sub> can block immune activation by CpG-ODNs. Short, 11–15 base long oligonucleotides were synthesized that were capable of potently inhibiting CpG-stimulation. The optimal inhibitory DNA motif consists of a pyrimidine-rich triplet, preferably CCT, which is positioned 5- to the GGG sequence in a singlestranded DNA molecule.
- Additionally, both the optimal spacing between the CCT and GGG motifs, as well as their relative order to each other, is of crucial importance for the inhibitory DNA action. Interestingly, although both TLR7/TLR8 ligands and bacterial DNA share the endosomal compartment for receptor binding and signal transduction, certain iODNs (G-type) suppress only TLR9-mediated activation, whereas prototype class I iODN may also interfere with the activation via the TLR7/TLR8 pathway.
- Recently, intriguing evidence has been presented that for some iODN classes the immunomodulatory biological activity shows only limited sequence dependency or may not even involve TLR-mediated uptake and signalling pathways. For example iODNs of the class II are thought to act on immune activation through inhibition of STAT signalling and independent of TLR signalling via binding to a yet to be identified “ODN-receptor”. Slightly modified phosphodiester versions of the most potent inhibitory ODNs were also able to profoundly block the immune activation of macrophages and just recently prove to be valuable tools for in vivo use in experimental animal models of inflammatory and auto-immune diseases.
- Based upon these recent insights the following **classification for iODNs** has been suggested:
  - **Class I:** G-stretch ODNs:TLR9-specific competitors
  - **Class II:** ODNs with telomeric repeats:TLR-independent inhibitors of STAT signalling
  - **Class III:** Inhibitors of DNA uptake in a sequence independent manner
  - **Class IV:** Long phosphorothioate ODNs as direct competitors of TLR9 signalling in a sequence independent manner

#### General Information

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- [1] *A TLR7 antagonist restricts interferon-dependent and -independent immunopathology in a mouse model of severe influenza.* Rappe JCF, et al. *J. Exp. Med.* (2021); 218:e20201631
- [2] *DNA Motifs suppressing TLR9 responses.* Trieu A, et al. *Crit. Rev. Immunol.* (2006); 26:527
- [3] *Inhibitory oligodeoxynucleotides-therapeutic promise for systemic autoimmune diseases?* Lenert P, *Clin. Exp. Immunol.* (2005); 140:1
- [4] *Immunotherapeutic utility of stimulatory and suppressive oligodeoxynucleotides.* Ishii KJ, et al. *Curr. Opin. Mol. Ther.* (2004); 6:166
- [5] *Suppressive oligodeoxynucleotides protect mice from lethal endotoxic shock.* Shirota H, et al. *J. Immunol.* (2005); 174:4579
- [6] *Toll-like receptor 9 inhibition reduces mortality in polymicrobial sepsis.* Plitas G, et al. *J. Exp. Med.* (2008); 205:1277

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