



## PRODUCT DATA SHEET

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### ODN 1668 (Type B) Endotoxin-free (sterile)

**Cat. No.:** IAX-200-001

**Lot. No.:**

|                       |   |
|-----------------------|---|
| <b>Sequence</b>       | 5'-tccatgacggttcctgatgct-3' (lower case letters: phosphorothioate linkage: nuclease resistant)  |
| <b>Synonyms</b>       | CpG ODN TLR9 Agonist: B Type (species: rodent)  |
| <b>MW</b>             | 6,383 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>       | Optimal concentration is dependent upon cell type, species, desired activation and analysis: <i>in vitro</i> 0.1-1µM (~0.6-6.0µg/ml); <i>in vivo</i> 50-250µg in rodent animal models.<br>Negative control: GpC ODN 1720 (Cat. No.: IAX-200-200). As an alternative control without agonistic nor antagonistic activity consider CTRL2-ODN (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-200-001 | **Version:** 1.5 | **Issue Date:** 30/01/2023

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#### Product Specific References

- [1] *Modulation of the Innate Immune Response by Targeting Toll-like Receptors: A Perspective on Their Agonists and Antagonists.* Federico F, et al. J. Med. Chem. (2020); 63:13466
- [2] *Small Molecule NF-κB Inhibitors as Immune Potentiators for Enhancement of Vaccine Adjuvants.* Moser BA, et al. Front. Immunol. (2020); 11:1
- [3] *p38α has an important role in antigen cross-presentation by dendritic cells.* Zhou Y, et al. Cellular & Molecular Immunology (2018); 15:246

#### General Information

- Unmethylated CG dinucleotides within particular sequence contexts are responsible for the immunostimulatory activity of bacterial DNA. Synthetic oligonucleotides (ODN) that contain such **CpG motifs (CpG ODNs)** mimic microbial DNA.
- The innate immune system of vertebrates has the ability to recognize CpG motifs in microbial DNA via the Toll-like receptor (TLR) 9 if the CpG ODN were free of additional immune stimulatory contaminants often present in synthetic commercial CpG ODN preparations designed for molecular biology applications (i.e. PCR). Given that high quality CpG ODNs were used [i.e. endotoxin-free], a close link has been established between the expression of TLR9 on certain immune cell subsets and the modulation of the immune system by CpG DNA.
- Different types of **CpG ODNs** were identified based on their differing biological effects on different cell types:
  - ODN **Type A** is a potent inducer of IFN-α in human PDC, (i.e. ODN 1585 or 2216) leading to antigen presenting cell (APC) maturation.
  - ODN **Type B** (i.e. ODN 2006 or ODN 1668/ODN 1826) is a weak inducer of IFN-α but rather stimulates production and increasing costimulatory and Ag-presenting molecules and triggers proliferation of B-cells, IgM, and IL-6 production.
  - A third type of CpG ODN has been identified, termed ODN **Type C**, with both high induction of INF-α in PDC and activation of B-cells. The sequence of CpG Type C (also called K) (i.e. ODN 2395 or M362) combines elements of both Type A and Type B and contain a central palindromic sequence with CG dinucleotides, a characteristic feature of Type A, and a 'TCGTCTG' motif at the 5' end, present in Type B CpG ODNs.
- Although the CpG motifs are thought to differ between mice and humans, in both species the recognition of CpG ODNs is mediated by TLR9. The optimal CpG motif in humans is GTCGTT and GACGTT for the murine sequence. However, recent evidence suggests that this sequence specificity is restricted to phosphorothioate (PS)-modified ODN and is not observed when a natural phosphodiester backbone is used. In recent years sequence requirements, specificity, signalling pathways and kinetics of the TLR9 suppression by '**inhibitory ODNs**' (**iODNs**) have been investigated.

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- [1] Comparison of different CpG oligodeoxynucleotide classes for their capability to stimulate human NK cells. Sivori S, et al. Eur. J. Immunol. (2006); 36:961
- [2] Innate immune responses induced by classes of CpG oligodeoxynucleotides in ovine lymph node and blood mononuclear cells. Booth JS, et al. Vet. Immunol. Immunopathol. (2007); 115:24
- [3] Impact of class A, B and C CpG-oligodeoxynucleotides on in vitro activation of innate immune cells in human immunodeficiency virus-1 infected individuals. Martinson JA, et al. Immunology (2007); 120:526
- [4] Three CpG oligodeoxynucleotide classes differentially enhance antigen-specific humoral and cellular immune responses in mice. Liu Y, et al. Vaccine (2011); 29:5778
- [5] Toll-like receptor and IL-12 signaling control susceptibility to contact hypersensitivity. Martin SF, et al. J. Exp. Med. (2008); 205:21
- [6] DNA Motifs suppressing TLR9 responses. Trieu A, et al. Crit. Rev. Immunol. (2006); 26:527
- [7] Nucleic acids of mammalian origin can act as endogenous ligands for Toll-like receptors and may promote systemic lupus erythematosus. Barrat FJ, et al. J. Exp. Med. (2005); 202:1131
- [8] 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

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