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PRODUCT DATA SHEET

TLR9 Agonist (human) Explorer Set Endotoxin-free

Cat. No.: IAX-300-007-1 **Date:** 21-Jan-2013

CONTENT: ODN 2216 (Type A) Endotoxin-free (sterile), 100µg (Cat. No.: IAX-200-005-C100).

ODN 2006 (Type B) Endotoxin-free (sterile), 100µg (Cat. No.: IAX-200-006-C100). ODN 2395 (Type C) Endotoxin-free (sterile), 100µg (Cat. No.: IAX-200-007-C100).

ddWater Endotoxin-free (sterile), 3x1.5ml (Cat. No.: IAX-900-002-LD15).

ENDOTOXIN-FREE: <0.002EU/μg.

HANDLING: Prepare a 100µM stock solution just prior to use, keep sterile and use only endotoxin-free and sterile ddWater

(Cat. No.: IAX-900-002), or PBS (Cat. No.: IAX-900-001).

ACTIVITY: TLR9 Agonists (species: human and rodent). Optimal concentration is dependent upon cell type, species, desired activa-

tion and analysis: *in vitro* 0.1-1µM; *in vivo* 50-250µg in rodent animal models. Negative control for ODN 2216: GpC ODN 2243 (Cat. No.: IAX-200-205). Negative control for ODN 2006: GpC ODN 2137 (Cat. No.: IAX-200-206). Negative control for ODN 2395: Neutral-ODN (Cat. No.: IAX-200-202).

SHIPPING: Ambient.

STORAGE: 4°C. After reconstitution, prepare aliquots and keep aqueous stock solutions for 1 day at 4°C or store at -20°C (shelf-life 6

months). Avoid freeze/thaw cycles.

STABILITY: 2 years after receipt as supplied.

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, whereas ODN **Type B** (i.e. ODN 2006 or ODN 1668 / ODN 1826) is a weak inducer of IFN- α but rather stimulates IL-8 production and increasing costimulatory and Ag-presenting molecules and triggers proliferation of B-cells and 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 'TCGTCG' 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.

References:

- [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); 205:2151

DISCLAIMER: THIS PRODUCT IS NOT INTENDED OR APPROVED FOR HUMAN, DIAGNOSTICS OR VETERINARY USE. USE OF THIS PRODUCT FOR HUMAN OR ANIMAL TESTING MAY BE EXTREMELY HAZARDOUS AND MAY RESULT IN DISEASE, SEVERE INJURY, OR DEATH. THIS PRODUCT IS FOR RESEARCH USE ONLY (RUO).

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.