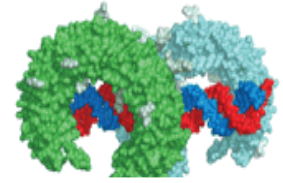


PRODUCT DATA SHEET

Duo-iODN (inhibitory ODN) Endotoxin-free (sterile)

Cat. No.: IAX-200-055

Date: 08-Jan-2013



SEQUENCE:	5'-tgctcctggaggggtgt-3' (lower case letters: phosphorothioate linkage: nuclease resistant).
SYNONYMS:	Inhibitory ODN (iODN): Class I.
MW:	5,867g/mol.
FORMULATION:	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).
ENDOTOXIN-FREE:	<0.002EU/µg.
HANDLING:	Reconstitution: For a 100µM stock solution, dissolve total vial content in 1,700µl (1mg size) or 170µl (100µg size) sterile endotoxin-free water or PBS. Add 50% of solvent and let dissolve for 10min. Add remaining 50% of the solvent and mix thoroughly. Moderate warming may aid dissolving.
ACTIVITY:	Potent sequence of an inhibitory ODN for <i>in vivo</i> use in rodents (50-150ug per injection): prototype class I that also inhibits TLR7 signalling. Negative control: 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:

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 (TTAGG)_n can block immune activation by CpG-ODNs. Short, 11–15 base long oligonucleotides were synthesized that were capable of potentially 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 immuno-modulatory 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

References:

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

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.