

## CpG oligodeoxynucleotides (or CpG ODNs) as Vaccine Adjuvant and ELISA kits

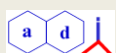
**CpG oligodeoxynucleotides (or CpG ODN)** are short single-stranded synthetic DNA molecules that contain an unmethylated CG (Cytosine–guanine) di nucleotide in a specific base sequence (CpG motifs). The p refers to the phosphodiester backbone. CpG oligodeoxynucleotides (ODNs) represent a novel pharmacotherapeutic class with profound immunomodulatory properties. These CpG motifs are not seen in eukaryotic DNA, in which CG dinucleotides are suppressed and, when present, usually methylated, due to this they are considered pathogen-associated molecular patterns (PAMPs). The CpG PAMP is recognized by the pattern recognition receptor (PRR) Toll-Like Receptor 9 (TLR9), which is constitutively expressed only in B cells and plasmacytoid dendritic cells (pDCs) in humans and other higher primates. Unmethylated CpG ODNs (18–24 bp in length) possess immunomodulatory properties similar to bacterial DNA and they act as immuno stimulants. The immunostimulatory properties of CpGs include induction of a Th1-type response with prominent release of IFN- $\alpha$ , IL-12, and IL-18.

Synthetic CpG have a partially or completely phosphorothioated backbone instead of the typical phosphodiester backbone and a poly G tail at the 3' end, 5' end, or both. Numerous sequences have been shown to stimulate TLR9 with variations in the number and location of CpG dimers, as well as the precise base sequences flanking the CpG dimers. CpG ODNs are classified into 5 classes, based on their sequence, secondary structures, and effect on human peripheral blood mononuclear cells (PBMCs). The five classes are **Class A (Type D)**, **Class B (Type K)**, **Class C**, **Class P**, and **Class S**. Three major classes of immunostimulatory CpG-ODNs are well characterized according to their in vitro activities and chemical compositions. **Class A** stimulate the production of large amounts of Type I interferons, the most important one being IFN $\alpha$ , and induce the maturation of pDCs. They are also strong activators of NK cells through indirect cytokine signaling. **Class B** ODN are strong stimulators of human B cell and monocyte maturation. They stimulate the maturation of pDC to a lesser extent than Class A ODN and very small amounts of IFN $\alpha$ . **Class C** ODN combine features of both types A and B. They contain a complete phosphorothioate backbone and a CpG-containing palindromic motif. They induce strong IFN- $\alpha$  production from pDC and B cell stimulation.

Several groups have studied the sequence requirements, specificity, signaling pathways and kinetics of the TLR (Toll-like receptor) 9 suppression by **'inhibitory DNA motifs'**, which led to a revised classification of **inhibitory ODNs**. **Class I: G-stretch ODNs:** TLR9-specific competitors, some iODNs may also affect TLR7 and TLR8 signaling. **Class II: ODNs with telomeric repeats:** TLR-independent inhibitors of STAT signaling (cellular uptake via an "ODN receptor"?) **Class III: Inhibitors of DNA uptake** in a sequence independent manner **Class IV: Long phosphorothioate ODNs** as direct competitors of TLR9 signaling in a sequence independent manner.

### Product ordering Information (Data sheets and Prices are posted at the website)

Items	Description	TLR Type	Unlabeled Cat #	Biotin Conj. Cat #	Negative Control*
ODN1585	ODN 1585-Type A murine TLR9 agonist, Antigen grade; 5'-ggGGTCAA <b>CG</b> TTGAgggggg-3' (20 mer)	Murine TLR9 agonist	<a href="#">ODN1585-1</a> <a href="#">ODN1585-5</a>	ODN1585-B	ODN1585-1NC ODN1585-5NC
ODN 1668	ODN 1668- Type B murine TLR9 Agonist, Antigen grade; 5'-tccatgacgttcctgatgct-3' (20 mer)	Murine TLR9 agonist	<a href="#">ODN1668-1</a> <a href="#">ODN1668-5</a>	ODN1668-B	ODN1668-1NC ODN1668-5NC
ODN 1826	ODN 1826- Type B murine TLR9 Agonist, antigen grade; 5'-tccatgacgttcctgacgtt-3' (20 mer)	Murine TLR9 agonist	<a href="#">ODN1826-1</a> <a href="#">ODN1826-5</a>	ODN1826-B	ODN1826-1NC ODN1826-5NC
ODN 2006	ODN 2006 -Type B-human TLR9 agonist, antigen grade; 5'- <b>tcgctg</b> ttttgctg <b>ttttgctg</b> tt-3' (24 mer)	Human TLR9 agonist	<a href="#">ODN2006-1</a> <a href="#">ODN2006-5</a>	ODN2006-B	ODN2006-1NC ODN2006-5NC
ODN 2007	ODN 2007-Type B bovine/porcine TLR9 agonist, antigen grade; 5'- <b>tcgctg</b> ttgtcg <b>ttttgctg</b> tt-3' (22 mer)	Bovine TLR9 agonist	<a href="#">ODN2007-1</a> <a href="#">ODN2007-5</a>	ODN2007-B	ODN2007-1NC ODN2007-5NC
ODN 2216	ODN 2216-Type A human TLR9 Agonist, antigen grade, 5'-ggGGGA <b>CGATCGTC</b> gggggg-3'(20 mer)	Human TLR9 agonist	<a href="#">ODN2216-1</a> <a href="#">ODN2216-5</a>	ODN 2216-B	ODN 2216-1NC ODN 2216-5NC
ODN 2336	ODN 2336-Type A human specific TLR 9 agonist, antigen grade; 5'-gggGA <b>CGACGTCGT</b> Gggggg -3' (21 mer)	Human TLR9 agonist	<a href="#">ODN2336-1</a> <a href="#">ODN2336-1</a>	ODN2336-B	ODN2336-1NC ODN2336-5NC
ODN 2395	ODN 2395-Type C human/murine TLR9 agonist, antigen grade. 5' <b>tcgctg</b> tttcg <b>cgcgcg</b> cg-3' (22 mer)	Human TLR9 agonist	<a href="#">ODN2395-1</a> <a href="#">ODN2395-5</a>	ODN2395-B	ODN2395-1NC ODN2395-1NC
ODN M362	ODN M362-Type C human/murine TLR9 agonist, antigen grade; 5'- <b>tcgctg</b> ctg <b>tcgacg</b> agcttgat-3' (25 mer)	Human TLR9 agonist	<a href="#">ODNM362-1</a> <a href="#">ODNM362-1</a>	ODNM362-B	ODNM362-1NC ODNM362-5NC
AT-ODN-1	AT ODN-Non-CpG AT rich ODN. TLR9 agonist, Antigen grade. 5'-tata <b>at</b> ttta <b>at</b> tccaaga-3' (20 mer)	TLR9 agonist	<a href="#">ATODN1-1</a>	4	4
AT-ODN-2	AT ODN-Non-CpG AT rich ODN. TLR9 agonist, Antigen grade 5'-tata <b>at</b> ttta <b>at</b> ccaactagc-3' (22 mer)	TLR9 agonist	<a href="#">ATODN2-1</a>	4	4
AT-ODN-2	AT ODN-Non-CpG AT rich ODN. TLR9 agonist, Antigen grade; 5'-taaca <b>at</b> ttta <b>at</b> ccaaga-3' (22 mer)	TLR9 agonist	<a href="#">ATODN3-1</a>	4	4
Neutral ODN	Control for inhibitory ODNs. No agonistic or Antagonist activity, Antigen grade; 5'-tgctctggagggggtgt-3' (18 mer)	Control	<a href="#">ODN-NT-1</a>	4	4
ODN BW006	ODNBW006 CpG ODN (contains structure type B sequence at 5' and Type A at the 3' end), antigen grade; 5'- <b>tcgacgtt</b> <b>tcgctg</b> tc <b>tcgctg</b> ttc-3' (23 mer)		<a href="#">ODN006-1</a>	4	4



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Items	Description	TLR Type	Unlabeled Cat #	Biotin Conj. Cat #	Negative Control*
ODN 2088	ODN 2088- Class I Murine TLR9 Antagonist, antigen grade; 5'-tcctgccggggaagt-3' (15 mer)	Murine TLR9 Antagonist	<a href="#">ODN2088-1</a> <a href="#">ODN2088-1</a>	4	ODN2088-1 ODN2088-1
ODN 4084-F	ODN 4084-Type B Inhibitory TLR9 Antagonist, antigen grade; 5'-cctggatgggaa-3' (15 mer)	TLR9 Antagonist	<a href="#">ODN4084F-1</a> <a href="#">ODN4084F-5</a>	4	ODN4084F-1 ODN4084F-5
ODN INH-1	ODN INH-1 -Class R (restricted) inhibitory ODN-TLR 9 Antagonist, antigen grade; 5'-cctggatgggaattcccatccagg-3' (23mer)	TLR9 Antagonist	<a href="#">ODNIHN1-1</a>	4	4
ODN INH-47	ODN INH-47- Class R (restricted) inhibitory ODN-TLR 9 Antagonist, antigen grade, 5'-tatggatttaataaaatccata-3' (23mer)	TLR9 Antagonist	<a href="#">ODNINH47-1</a>	4	4
ODN TTAGGG	ODN TTAGGG-Inhibitory ODN Human TLR9 antagonist, Antigen grade; 5'-tttagggttagggttagggttagg-3' (25 mer)	Human TLR9 antagonist	<a href="#">ODNTT-1</a>	4	ODNTT-1NC
G-ODN	G-ODN Murine TLR9 antagonist, Antigen grade; 5'-ctcctattgggggttcctat-3' (21 mer)	Murine TLR9 antagonist	<a href="#">GODN-1</a>	4	4
Core-iODN	Inhibitory ODN class I prototype (TLR7 and TLR8), antigen grade; 5'-tcctggagggg-3' (11mer)	TLR9 and TLR7 antagonist	<a href="#">CIODN-1</a>	4	4
Super-iODN	Inhibitory ODN- class I/II hybrid (TLR7 and TLR8), antigen grade, 5'-cctcaatagggtgagggg-3' (18mer)	TLR9 and TLR7 antagonist	<a href="#">SIODN-1</a>	4	4
Dual-iODN	Inhibitory ODN-prototype class I (TLR9 and TLR7 antagonist), antigen grade; 5'-tgctcctggaggggtgt-3' (18mer)	TLR9 and TLR7 antagonist	<a href="#">DIODN-1</a>	4	4

**Notes:** 1. Bases in capital are phosphodiester and those in lower case are phosphorothioate. Palindromic sequences are underlined. 2. Bases depicted in italics show AT-ODN sequence. 3. Negative control contains GpC nucleotides instead of CpG. 4. Contact ADI to place a special order for Biotin or FITC Conjugates.

### ELISA Kits for the study of Adjuvant effect on antibody response

The main function of adjuvants (conventional oil-based, special formulation or ODNs) is to enhance antibody response to a given antigen. The antibody levels or titer in the host species (mouse, rat, rabbit, G. pig, Ferret, Hamster, monkey or human etc) is typically measure by ELISA. ADI provides several species specific [ELISA kits to measure antibody titer](#). These kits contain all necessary reagents, except user's specific antigen, for about 1000 tests. Please see details at:

[http://4adi.com/commerce/catalog/spcategory.jsp?category\\_id=2473](http://4adi.com/commerce/catalog/spcategory.jsp?category_id=2473)

ADI also has ELISA kit for IgG, IgM, IgA, IgE or individual IgG isotypes (IgG1, 2a, 2b, IgG3) in serum or plasma for mouse, rat, human etc.

[http://4adi.com/commerce/catalog/spcategory.jsp?category\\_id=2446](http://4adi.com/commerce/catalog/spcategory.jsp?category_id=2446)

### ELISA Kits for the study of Adjuvant effect on antibody response using model antigens

Several model proteins have been used as model antigens (Bovine serum albumin/BSA or HSA, ovalbumin or OVA, Keyhole limpet hemocyanins/KLH, thyroglobulin, DNP-KLH/Albumin or Tetanus Toxoid or TTX or Diphtheria Toxoids. Adjuvants (conventional or ODN-based) have been used to study antibody response to a model antigen in a given species of host. ADI has specific antibody ELISA kits for these model antigens. Please see available kits at:

[http://4adi.com/commerce/catalog/spcategory.jsp?category\\_id=2719](http://4adi.com/commerce/catalog/spcategory.jsp?category_id=2719)

[http://4adi.com/commerce/catalog/spcategory.jsp?category\\_id=2449](http://4adi.com/commerce/catalog/spcategory.jsp?category_id=2449)

### ELISA Kits to measure ODN-induced anti-dsDNA or anti-ssDNA antibody

It is possible that some DNA-sequences (ODNs) when used as adjuvant may invoke anti-DNA antibodies. The presence of absence of anti-DNA antibodies must be investigated if any ODNs is intended for vaccines.

[http://4adi.com/commerce/catalog/spcategory.jsp?category\\_id=2448](http://4adi.com/commerce/catalog/spcategory.jsp?category_id=2448)

ODN\_Vaccine\_Flr 130207A

