

# ODN 1826 Ready-to-use

Order no. 130-109-374 Order no. 130-109-373

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# 1. Description

This product is for research use only.

Components	<b>130-109-374:</b> 5× 100 μg ODN in 50 μL sterile, physiological NaCl solution
	or
	<b>130-109-373:</b> 20× 100 μg ODN in 50 μL sterile, physiological NaCl solution.
Description	Murine B-class CpG oligodeoxyribonucleotide (ODN).
Product format	Fully reconstituted without carrier protein or preservatives. Sterile filtered; always handle under aseptic conditions.
Sequence	dT*dC*dC*dA*dT*dG*dA*dC*dG*dT*dT* dC*dC*dT*dG*dA*dC*dG*dT*dT * Phosphorothioate backbone
Endotoxin level	Low endotoxin (<1 EU/mg) as determined by kinetic Limulus Amebocyte Lysate (LAL) assay.
Storage	Store in aliquots at -20 °C. Aliquots are stable for 6 months. Avoid repeated freeze-thaw cycles. The expiration date is indicated on the vial label.

# 2. Background information

TLR9 is a prominent member of the toll-like-receptor (TLR) family recognizing pathogen-associated molecular patterns. TLR9 recognizes specifically unmethylated CpG motifs in bacterial DNA leading to activation of immune cells<sup>1,2</sup>. These effects can be mimicked by short synthetic ODNs containing unmethylated CpG motifs<sup>3</sup>. Several classes of CpG ODNs have been identified and can be distinguished by their effects on certain cell types<sup>4</sup>. A-class ODNs containing 5' and 3' G-rich stretches induce high levels of type I IFN but show low induction of B cell proliferation<sup>5</sup>. B-class ODNs activate B cells and TLR9-dependent

NF-KB signaling in recombinant cell lines but show low induction of IFN-a. C-class ODNs induce high amounts of IFN-a and activate B cells6. The recently discovered P-Class ODNs show similar but superior properties to C-class ODNs.7

ODN 1826 Ready-to-use is a B-class ODN that strongly activates murine TLR9.

# 3. Applications

#### 3.1 General applications

- CpG ODNs can be used for activation of immune cells, such as human peripheral blood mononuclear cells (PBMCs), murine splenocytes, or isolated immune cells (e.g., B cells and plasmacytoid dendritic cells).
- CpG ODNs can be used to activate signaling in TLR9expressing recombinant cell lines.

#### 3.2 Specific applications

Murine B-class ODNs have been extensively used as adjuvant for in vivo vaccination strategies to improve vaccine-specific antibody<sup>5</sup> responses via strong activation of TLR9 in mice<sup>8-11</sup>. ODN 1826 has been shown to function as a very efficient adjuvant alone<sup>11-13</sup> or in combination with other types of adjuvant<sup>11,14-16</sup> via different routes of administration<sup>10</sup>.

## 4. Instructions for use

After thawing vortex the solution and spin down.

100 µg ODN in 50 µL sterile, physiological NaCl solution is equivalent to a concentration of  $314 \ \mu M$ .

The working concentration in mice ranges from 10-20 µg<sup>11,12,14,15</sup> to 50  $\mu$ g<sup>12</sup> or up to 100  $\mu$ g<sup>14</sup>.

Recommended concentrations for cell culture use are

for murine immune cells: 0.05-2 µM for recombinant cell lines: 0.05–10  $\mu M$ 

▲ An excessively high concentration of ODNs may result in decreased activity. Therefore, the optimal concentration range should be determined for individual assay systems.

## 5. References

- 1. Hemmi, H. et al. (2000) A Toll-like receptor recognizes bacterial DNA. Nature 408:740-745
- 2 Krieg, A. M. et al. (1995) CpG motifs in bacterial DNA trigger direct B-cell activation. Nature 374: 546-549.
- Bauer, S. et al. (2001) Human TLR9 confers responsiveness to bacterial DNA 3. via species-specific CpG motif recognition. Proc. Natl. Acad. Sci. U.S.A. 98: 9237-9242.

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- Vollmer, J. and Krieg, A. M. (2009) Immunotherapeutic applications of CpG oligodeoxynucleotide TLR9 agonists. Adv. Drug Deliv. Rev. 61: 195–204.
- Krug, A. S. et al. (2001) Identification of CpG oligonucleotide sequences with high induction of IFN-alpha/beta in plasmacytoid dendritic cells. Eur. J. Immunol. 31: 2154–2163.
- Vollmer, J. et al. (2004) Characterization of three CpG oligodeoxynucleotide classes with distinct immunostimulatory activities. Eur. J. Immunol. 34: 251–262.
- Samulowitz, U. *et al.* (2010) A novel class of immune-stimulatory CpG oligodeoxynucleotides unifies high potency in type I interferon induction with preferred structural properties. Oligonucleotides 20: 93–101.
- Klinman, D. M. (2006) Adjuvant activity of CpG oligodeoxynucleotides. Int. Rev. Immunol. 25: 135–154.
- McCluskie, M. J. and Weeratna, R. D. (2001) Novel adjuvant systems. Curr. Drug Targets Infect. Disord. 1: 263–271.
- McCluskie, M. J. and Davis, H. L. (2001) Oral, intrarectal and intranasal immunizations using CpG and non-CpG oligodeoxynucleotides as adjuvants. Vaccine 19: 413–422.
- 11. Weeratna, R. D. *et al.* (2000) CpG DNA induces stronger immune responses with less toxicity than other adjuvants. Vaccine 18: 1755–1762.
- Coban, C. et al. (2004) Effect of CpG Oligodeoxynucleotides on the immunogenicity of Pfs25, a Plasmodium falciparum transmission-blocking vaccine antigen. Infection and Immunity 72 (1): 584–588.
- Al-Mariri, A. *et al.* (2001) Protection of BALB/c mice against *Brucella abortus* 544 challenge by vaccination with bacterioferritin or P39 recombinant proteins with CpG oligodeoxynucleotides as adjuvant. Infection and Immunity 69 (8): 4816–4822.
- 14. Chu, R. *et al.* (1997) CpG oligodeoxynucleotides act as adjuvants that switch on T helper 1 (TH1) immunity. J. Exp. Med. 186 (10): 1623–1631.
- Brazolot, C. L. *et al.* (1998) CpG DNA can induce strong TH1 humoral and cellmediated immune responses against hepatitis B surface antigen in young mice. Proc. Natl. Acad. Sci. U.S.A. 95: 15553–15558.
- Kumar, S. *et al.* (2004) CpG oligodeoxynucleotide and Montanide ISA 51 adjuvant combination enhanced the protective efficacy of a subunit malaria vaccine. Infect. Immun. 72: 949–957.

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