Miltenyi Biotec

# TLR9 Explorer

Order no. 130-100-589

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## 1. Description

**Components** **1 mL 1× TE Buffer**
**8×100 µg lyophilized ODNs**

For details refer to table below:

| Component | Content | Class / Sequence |
|---|---|---|
| ODN 2006 | 100 µg | B-Class CpG Oligodeoxyribonucleotide<br>dT*dC*dG*dT*dC*dG*dT*dT*dT*dT*dG*dT*dC*dG*dT*dT*dT*dT*dG*dT*dC*dG*dT*dT |
| ODN 2006 Control (ODN 2137 ) | 100 µg | B-Class CpG Control Oligodeoxyribonucleotide<br>dT*dG*dC*dT*dG*dC*dT*dT*dT*dT*dG*dT*dG*dC*dT*dT*dT*dT*dG*dT*dG*dC*dT*dT |
| ODN 2395 | 100 µg | C-Class CpG Oligodeoxyribonucleotide<br>dT*dC*dG*dT*dC*dG*dT*dT*dT*dT*dC*dG*dG*dC*dG*dC*dC*dG*dC*dG*dC*dC*dG |
| ODN 2395 Control (ODN 5328) | 100 µg | C-Class CpG Control Oligodeoxyribonucleotide<br>dT*dG*dC*dT*dG*dC*dT*dT*dT*dT*dC*dG*dG*dC*dG*dC*dG*dC*dG*dC*dC*dG |
| ODN 21798 | 100 µg | P-Class CpG Oligodeoxyribonucleotide<br>dT*dC-dG*dT*dC-dG*dA*dC-dG*dA*dT*dC-dG*dG*dC*dG*dC-dG*dC*dG*dC*dC* dG |
| ODN 21798 Control (ODN 23098) | 100 µg | P-Class CpG Control Oligodeoxyribonucleotide<br>dT*dG-dC*dT*dC-dG*dA*dC-dG*dA*dT*dC-dG*dG*dC*dG*dC-dG*dC*dG*dC*dC* dG |
| ODN 2216 | 100 µg | A-Class CpG Oligodeoxyribonucleotide<br>dG*dG*dG-dG-dG-dA-dC-dG-dA-dT-dC-dG-dT-dC-dG*dG*dG*dG*dG*dG |
| ODN 2216 Control (ODN 2243) | 100 µg | A-Class CpG Control Oligodeoxyribonucleotide<br>dG*dG*dG-dG-dG-dA-dG-dC-dA-dT-dG-dC-dT-dC-dG*dG*dG*dG*dG*dG |

* Phosphorothioate backbone

**Product format** Lyophilized product without carrier protein or preservatives.

**Endotoxin level** Low endotoxin (<1 EU/mg) as determined by kinetic Limulus Amebocyte Lysate (LAL) assay.

**Storage** Store lyophilized product at –20 °C. Upon reconstitution, aliquots should be stored at –20 °C and 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[1,2]. These effects can be mimicked by short synthetical ODNs containing unmethylated CpG motifs[3]. Several classes of CpG ODNs have been identified and can be distinguished by their effects on certain cell types[4]. A-class ODNs containing 5' and 3' G-rich stretches induce high levels of type I IFN but show low activation of B cell proliferation[5]. B-class ODNs activate B cells and TLR9-dependent NF-κB signaling in recombinant cell lines but show low induction of IFN-α. C-class ODNs induce high amounts of IFN-α and activate B cells[6]. The recently discovered new P-Class ODNs show similar but superior properties to C-class ODNs.[7]
Control compounds for CpG ODNs share the sequence with their stimulatory counterparts but contain GpC dinucleotides instead of the CpG motifs and thus control for the CpG specificity of the effects.

## 3. Applications

### 3.1 General applications

- CpG ODNs can be used for activation of immune cells, such as human PBMCs, murine splenocytes or isolated immune cells (e.g., B cells and pDCs).
- CpG ODNs can be used to activate signaling in TLR9-expressing recombinant cell lines.
- Control ODNs can be used as a sequence control for their respective sequence counterparts.

### 3.2 Specific applications

- A-class ODNs are useful for pDCs activation and IFN-α induction from human PBMCs and activation of IFN-signaling pathways. A-class ODNs form tertiary structures (G tetrads).
- B-class ODNs are useful for B cell activation and IL-6 induction from human PBMCs and activtion of NF-κB-signaling pathways.


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140-004-205.02

- C-class ODNs are useful for B cell activation and IFN-α induction. They activate B cells and pDCs. C-Class ODNs form dimeric structures.
- P-class ODNs activate both, B cells and pDCs with higher efficiency than C-class ODNs. P-class ODNs can form multimeric structures.

## 4. Instructions for use

### 4.1 Recommended concentrations

Recommended concentrations for use are

for human and murine immune cells: 0.05–2 µM

for recombinant cell lines: 0.05–10 µM

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

### 4.2 Reconstitution protocol

1. Spin down pellet.
2. Resuspend lyophilized ODN. The volume of 1× TE Buffer necessary to obtain a 200 µM solution is indicated in the table below.
   ▲ **Note:** Alternatively, PBS or water can be used for reconstitution.
3. Vortex and incubate overnight at 4 °C.
4. Store aliquots at –20 °C.

| Component | Resuspension volume for 100 µg |
|---|---|
| ODN 2006 | 65 µL |
| ODN 2006 Control (ODN 2137 ) | 65 µL |
| ODN 2395 | 71 µL |
| ODN 2395 Control (ODN 5328) | 71 µL |
| ODN 21798 | 68 µL |
| ODN 21798 Control (ODN 23098) | 68 µL |
| ODN 2216 | 78 µL |
| ODN 2216 Control (ODN 2243) | 78 µL |

## 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.
3. Bauer, S. *et al.* (2001) Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. Proc. Natl. Acad. Sci. U.S.A. 98: 9237–9242.
4. Vollmer, J. and A. M. Krieg (2009) Immunotherapeutic applications of CpG oligodeoxynucleotide TLR9 agonists. Adv. Drug Deliv. Rev. 61: 195–204.
5. Krug, A. *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.
6. Vollmer, J. *et al.* (2004) Characterization of three CpG oligodeoxynucleotide classes with distinct immunostimulatory activities. Eur. J. Immunol. 34: 251–262.
7. 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.


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