Miltenyi Biotec

ODN 2006

Order no. 130-100-106
Order no. 130-100-105

Contents

1. Description

Components	**130-100-106:** 200 µg lyophilized ODN 1 mL 1× TE Buffer or **130-100-105:** 1 mg lyophilized ODN 1 mL 1× TE Buffer.
Description	B-class CpG oligodeoxyribonucleotide (ODN).
Product format	Lyophilized product without carrier protein or preservatives.
Sequence	dT*dC*dG*dT*dC*dG*dT*dT*dT*dT*dG*dT* dC*dG*dT*dT*dT*dT*dG*dT*dC*dG*dT*dT * Phosphorothioate backbone
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 synthetic 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 P-Class ODNs show similar but superior properties to C-class ODNs.[7]

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.

3.2 Specific applications

- B-class ODNs are useful for B cell activation and IL-6 induction from human PBMCs and activation of NF-κB-signaling pathways.

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. a) For 200 µg lyophilized ODN:
   To obtain a 200 µM solution resuspend pellet in 130 µL of 1× TE Buffer.
   ▲ **Note:** Alternatively, PBS or water can be used for reconstitution.

   b) For 1 mg lyophilized ODN:
   To obtain a 200 µM solution resuspend pellet in 650 µL of 1× TE Buffer.
   ▲ **Note:** Alternatively, PBS or water can be used for reconstitution.
3. Vortex and incubate overnight at 4 °C.
4. Store aliquots at –20 °C.

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5. References

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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 Krieg, A. M. (2009) Immunotherapeutic applications of CpG oligodeoxynucleotide TLR9 agonists. Adv. Drug Deliv. Rev. 61: 195–204.
5. 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.
6. Vollmer, J. *et al.* (2004) Characterization of three CpG oligodeoxynucleotide classes with distinct immunostimulatory activities. Eur. J. Immunol. 34: 251–262.
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