Miltenyi Biotec

ORN R-1263

Order no. 130-104-433
Order no. 130-104-435

Contents

1. Description

Components	**130-104-433:** 200 µg lyophilized ORN 1 mL RNase-free Water 400 µL DOTAP-Chloride (1 mg/mL) or **130-104-435:** 1 mg lyophilized ORN 1 mL RNase-free Water 5×400 µL DOTAP-Chloride (1 mg/mL)
Description	TLR7 / TLR8 agonist control.
Product format	Lyophilized ORN without carrier protein or preservatives.
Sequence	rG*rC*rC*rA*rC*rC*rG*rA*rG*rC*rC*rG*rA* rA*rG*rG*rC*rA*rC*rC * 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. Store DOTAP-Cl at 2–8 °C. The expiration date is indicated on the vial label.

2. Background information

TLR7 and TLR8 are members of the Toll-like-receptor (TLR) family recognizing pathogen-associated molecular patterns.[1,7] TLR7 and 8 recognize (virally derived) double- or single-stranded RNA leading to activation of immune cells. These natural ligands can be mimicked by short synthetic RNAs (oligoribonucleotides; ORNs).[2-5] RNA motifs have been described activating either TLR7 or TLR8 only or both, TLR7 and TLR8, simultaneously. TLR7 and TLR8 are expressed on human cells with a distinct expression pattern. In human cells, pDCs and B cells express predominantly TLR7 whereas TLR8 is mainly expressed on cells of the myeloid lineage, such as monocytes, macrophages, and myeloid dendritic cells (mDCs). In mice, TLR7 is also expressed on mDCs and monocytes whereas murine TLR8 is functionally impaired.[1,7]
Due to the sensitive nature of RNA, most nucleic acid TLR7/8 agonists need to be formulated with cationic lipids in order to activate an appropiate immune response. The recommended cationic lipid is DOTAP-Cl (1,2-Dioleoyloxy-3-trimethylammonium-propane chloride).

3. Application

- ORN R-1263[3] can be used as a sequence and backbone control for ORN R-0002 and ORN R-0006.

4. Instructions for use

4.1 Recommended concentrations

Recommended concentrations for use are

for human and murine immune cells: 0.05–2 µM

▲ An excessively high concentration of ORNs 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 ORN:
   To obtain a 200 µM solution resuspend pellet in 149 µL of RNase-free Water.
   b) For 1 mg lyophilized ORN:
   To obtain a 200 µM solution resuspend pellet in 745 µL of RNase-free Water.
3. Vortex and incubate overnight at 4 °C.
4. Store aliquots at –20 °C.

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4.3 Recommended formulation protocol

For formulation of the ORNs with DOTAP-Cl we recommend to use a ratio of 2 µM ORN and 25 µg/mL DOTAP-Cl. The complexation of ORN with DOTAP-Cl should be performed in water, PBS or serum-free medium in a smaller volume (e.g., 1/10 of well volume). Always add DOTAP-Cl to the ORN solution. We recommend to keep this ratio constant if probing several ORN concentrations. For dilution series, ORN can be formulated at an initial concentration of 2 µM ORN and 25 µg/mL DOTAP-Cl. This complex can be diluted with medium or water to the desired concentrations.

Final concentration per well	Water	ORN (200 µM)	DOTAP-Cl (1 mg/mL)
2 µM ORN	8 µL	2 µL	
25 µg/mL DOTAP-Cl	5 µL		5 µL

Add 10 µL of diluted DOTAP-Cl to 10 µL of diluted ORN solution, mix by pipetting, and add to cells. Alternatively, further dilute solution to the desired concentration.

Example:

	per 96-well round bottom plate
Recommended cell density (for PBMCs)	1×10^6 cells/well
Cell culture medium	180 µL
ORN/DOTAP-Cl complex	20 µL
Total volume	**200 µL**

5. References

1. Blasius, A. L., and Beutler, B. (2010) Intracellular toll-like receptors. Immunity 32(3): 305–315.
2. Diebold, S. S. *et al.* (2004) Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science 303: 1529–1531.
3. Forsbach, A. *et al.* (2008) Identification of RNA sequence motifs stimulating sequence-specific TLR8-dependent immune responses. J. Immunol. 180: 3729–3738.
4. Forsbach, A. *et al.* (2011) Dual or triple activation of TLR7, TLR8, and/or TLR9 by single-stranded oligoribonucleotides. Nucleic Acid Ther. 21(6): 423–436.
5. Heil, F. *et al.* (2004) Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science 303: 1526–1529.
6. Jurk, M. *et al.* (2011) Immunostimulatory potential of silencing RNAs can be mediated by a non-uridine-rich toll-like receptor 7 motif. Nucleic Acid Ther. 21(3): 201–214.
7. Kawai, T., and Akira, S. (2010) The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat. Immunol. 11(5): 373–384.

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