Miltenyi Biotec

ORN R-0002

Order no. 130-104-427
Order no. 130-104-438

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1. Description

Components	**130-104-427:** 200 µg lyophilized ORN 1 mL RNase-free Water 400 µL DOTAP-Chloride (1 mg/mL) or **130-104-438:** 1 mg lyophilized ORN 1 mL RNase-free Water 5×400 µL DOTAP-Chloride (1 mg/mL)
Description	TLR8 agonist.
Product format	Lyophilized product without carrier protein or preservatives.
Sequence	rU*rU*rA*rU*rU*rA*rU*rU*rA*rU*rU*rA*rU* rU*rA*rU*rU*rA*rU*rU * 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. Applications

3.1 General application

- TLR7 and TLR8 agonists can be used for the activation of immune cells, such as human PBMCs, murine splenocytes, or isolated immune cells, e.g., B cells, pDCs, mDCs, and monocytes.

3.2 Specific applications

- ORN R-0002[3] activates specifically human TLR8 and induces a TLR8-biased spectrum of cytokines from human PBMCs (predominantly TNF-α, IFN-γ, IL-12).

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 154 µL of RNase-free Water.
   b) For 1 mg lyophilized ORN:
   To obtain a 200 µM solution resuspend pellet in 769 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

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