Miltenyi Biotec

TLR7/8 Explorer

Order no. 130-104-388

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1. Description

Components 5×100 µg lyophilized ORNs
1 mL RNase-free Water
3×400 DOTAP-Chloride (1 mg/mL)

For details refer to table below:

Component	Content	Application / Sequence
ORN R-0002	100 µg	TLR8 agonist, human rU*rU*rA*rU*rU*rA*rU*rU*rA*rU*rU*rA*rU*rU*rA*rU*rU*rA*rU*rU
ORN R-0006	100 µg	TLR7 agonist, human and mouse, TLR8 agonist, human rU*rU*rG*rU*rU*rG*rU*rU*rG*rU*rU*rG*rU*rU*rG*rU*rU*rG*rU*rU
ORN R-1263	100 µg	Control for ORN R-0002 and ORN R-0006 rG*rC*rC*rA*rC*rC*rG*rA*rG*rC*rC*rG*rA*rA*rG*rG*rC*rA*rC*rC
ORN R-2336	100 µg	TLR7 agonist, human and mouse rG-rA-rC-rA-rC-rA-rC-rA-rC-rA-rC-rU-rC-rA-rC-rA-rC-rA-rC-rA-rC-rA-rC-rA
ORN R-2336 Control	100 µg	Control for ORN R-2336 rG-rA-rC-rA-rC-rA-rC-rA-rC-rA-rC-rA-rC-rA-rC-rA-rC-rA-rC-rA-rC-rU
* Phosphorothioate backbone		
- Phosphodiester backbone		

Product format Lyophilized ORN products without carrier protein or preservatives

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

Storage Store lyophilized products 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

- The ORNs[3-5] contained in this kit activate both human TLRs, TLR7 and TLR8 as well as murine TLR7 and induce a broad spectrum of cytokines from human PBMCs (IFN-α, IFN-γ, IL-12, and TNF-α).
- Control ORN do not activate TLR7 or TLR8 and can serve to control for backbone- or DOTAP-related effects.

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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. Resuspend lyophilized ORNs. The volume of water necessary to obtain a 200 µM solution is indicated in the table below.
3. Vortex and incubate overnight at 4 °C.
4. Store aliquots at –20 °C.

Component	Resuspension volume for 100 µg
ORN R-0002	77 µL
ORN R-0006	76 µL
ORN R-1263	75 µL
ORN R-2336	66 µL
ORN R-2336 Control	66 µL

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.
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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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