# ORN RNA 41

Order no. 130-104-430
Order no. 130-104-448

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## 1. Description

| | |
|---|---|
| **Components** | **130-104-430:**<br>200 µg lyophilized ORN<br>1 mL RNase-free Water<br>400 µL DOTAP-Chloride (1 mg/mL)<br>or<br>**130-104-448:**<br>1 mg lyophilized ORN<br>1 mL RNase-free Water<br>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*rC*rG*rA*rC*rA*rG*rA*rA*rG*rA*rG*rA*rG*rA*rC*rA*rC<br>* 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.<br>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 RNA 41[5] can be used as a sequence and backbone control for ORN RNA 40 (# 130-104-428, # 130-104-429).

## 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 148 µL of RNase-free Water.
   b) For 1 mg lyophilized ORN:
   To obtain a 200 µM solution resuspend pellet in 738 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) | $1\times10^6$ cells/well |
| Cell culture medium | 180 µL |
| ORN/DOTAP-Cl complex | 20 µL |
| **Total volume** | **200 µL** |

## 5. References

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