riboxx
MAKE THE DIFFERENCE

Made in ISO 9001:2008 Germany

RIBOXXOL® green488

RNA duplex - TLR3 ligand coupled to a green chromophore

This datasheet (Version # 20160914) is valid for the following products:
Art.-No. A-00103-0001, Art.-No. A-00103-0002

For research and pre-clinical use only. Not intended for use in humans.

A. PRODUCT INFORMATION

1. Content

Lyophilized RIBOXXOL coupled to a green chromophore (dry material).
Endotoxin-free RNAse-DNAse free water.
RNase DNAse free sterile tube.

2. Storage

RIBOXXOL green488 is shipped as lyophilized material at room temperature.

Short term storage: Dissolve in endotoxin-free RNAse-DNAse free water and store at 4°C for max. 4 weeks. **Protect from light and store in the dark!**

Long term storage: Prepare aliquots of the solution and store at -20°C. **Avoid more than 3 x freeze-thaw cycles.**

3. Stability

Product is stable for 6 months when stored at -20°C.
Repeated freezing-thawing reduces stability of the product.

4. Quality control

RIBOXXOL green488 is a double-stranded RNA duplex consisting of cytosines, guanosines and inosines, coupled to a chromophore. The chromophore green488 is an analog of Cy2.
It is prepared under RNAse-DNAse free conditions and is certified endotoxin-free (< 1 EU/mg, measurement by kinetic chromogenic LAL assay).
The supplied RNAse-DNAse free water is certified endotoxin-free (< 1 EU/ml).

5. Chemical properties

CAS number: 63231-63-0
Ribonucleic acid duplex
Molecular weight: 35.5 KDa
Length: 50 bp
Base composition: Cytosines, inosines, guanosines
Chromophore: riboxx green488 (Abs./Em.= 488/505 nm)
Solubility: product is soluble up to a concentration of 2 mg/ml.
DO NOT HEAT the product to increase solubility as this may degrade it.
Working concentration: 1-2 µg/µl

B. DESCRIPTION

RIBOXXOL green488 is a synthetic double stranded RNA (dsRNA). It has a length of 50 bp coupled to a green chromophore. It is composed of cytosines, inosines and guanosines.

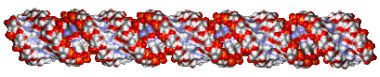

Fig. 1. Structural model of RIBOXXOL (RNA duplex, 50 bp).

Double stranded RNA is a potent activator of innate immunity. In the context of innate immunity, dsRNA is a pathogen associated molecular pattern (PAMP) that activates innate immune response through pathogen recognition receptors (PRR). The PRR of RIBOXXOL is Toll-like-receptor 3 (TLR3).

TLR3 is present in the endosome of myeloid dendritic cells (DCs) and Natural Killer cells [1]. Signaling of TLR3 is triggered by dsRNA with a length of more than 45 bp [2,3]. Triggering the TLR3-pathway through dsRNA induces IL-1ß, 1L-12 and type I IFNs production, improves cross-presentation of antigens and MHC class I expression. RIBOXXOL promotes Th1 (cellular) immune response, production of IFN-γ by NK cells, and activates monocytes.

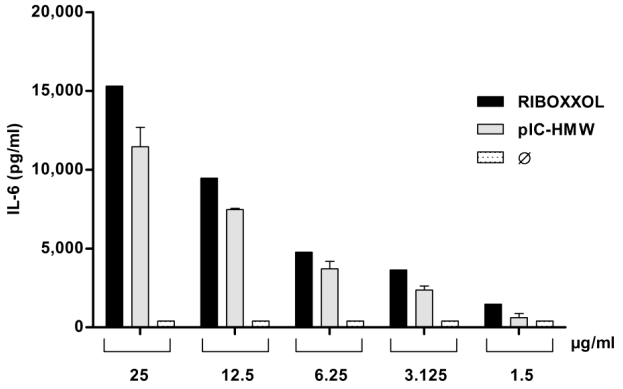

Fig. 2. Activation of human Dendritic Cells (DCs) by RIBOXXOL. *DCs were isolated from a blood donor and incubated with RIBOXXOL at the indicated concentrations. IL-6 was measured in the supernatant after 48h. As a comparison, poly(I:C) High Molecular Weight (pIC-HMW) was used. The results shown correspond to the MEAN+/- SEM of 3 independent measures.*

RIBOXXOL green488 is taken up by dendritic cells and localizes to the endosome (Fig. 3). In non-immune cells, RIBOXXOL green488 is taken up upon formulation with liposomes such as riboxxFECT. Fluorescence Intensity can be measured by flow cytometry.

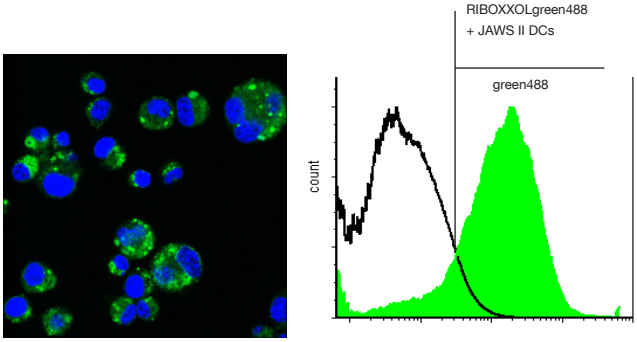

Fig. 3. Uptake of RIBOXXOL green488 by immune cells (JAWS II DCs). ***A,*** *RIBOXXOL green488 was used at a concentration of 5 µg/ml. Fluorescence intensity was visualized by confocal microscopy.* ***B,*** *RIBOXXOLgreen488 was used at a concentration of 5 µg/ml. Fluorescence intensity was visualized by flow cytometry.*

C. FIELDS OF APPLICATION

RIBOXXOL green488 is dedicated to be used in the following indications
- Mechanism of action studies of TLR3-ligands
- Uptake and tracking of TLR3-ligands in mammalian cells
- Flow cytometry studies in mammalian cells

D. METHODS

1. Preparation of RIBOXXOL green488 solution (1 µg/µl)

Protocol

- **IMPORTANT:** Before dissolving the product, perform a short spin at max. speed in a centrifuge to collect the pellet at the bottom of the RIBOXXOL green488 vial.
- Add RNAse-DNAse free water to the RIBOXXOL green488 vial.
- Mix the solution by pipetting up and down.
- **ATTENTION - DO NOT HEAT** the mixture as this may result in degradation!
- Up to a concentration of 2 µg/µl, the product dissolves within seconds. Solution should be clear without precipitates.

2. TLR3 ligation with RIBOXXOL green488

Ligation of TLR3 by RIBOXXOL green488 can be monitored using JAWS II dendritic cells. JAWs II DCs are murine immature myeloid dendritic cells. They have ben used in studies focusing on antitumor and pathogen-specific immunity [4] and are highly sensitive to TLR3 ligands [5]. Upon activation, JAWS II DCs secrete IL-6 in the supernatant that can be measured by ELISA.

Protocol

- Plate a JAWS II DCs cell suspension at 50,000 cells/well in a 96-well plate in DMEM with 10% fetal calf serum (FCS), and 1% penicillin/streptomycin (100 U / ml).
- Add 5-10 µg/ml RIBOXXOL per well and incubate for 16-24 h.
- Measure IL-6 concentration in supernatant by ELISA.

A typical result is shown in Fig. 4.

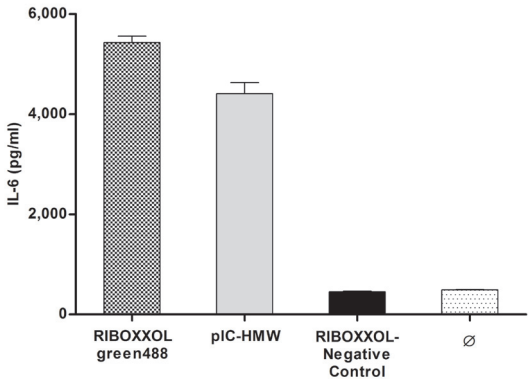

Fig. 4. Activation of JAWS II DCs by RIBOXXOL green488. *DCs were incubated with RIBOXXOL green488 at 12.5 µg/ml. After 24h, IL-6 was measured in the supernatant. As a comparison, poly(I:C) High Molecular Weight (pIC-HMW) was used at the same concentration. The results shown correspond to the MEAN+/- SEM of 3 independent measures.*

E. REFERENCES

1. Gay, N.J., et al., 2006. Toll-like receptors as molecular switches. Nat Rev Immunol 6, 693-8.

2. Jelinek, I., et al., 2011. TLR3-specific double-stranded RNA oligonucleotide adjuvants induce dendritic cell cross-presentation, CTL responses, and antiviral protection. J Immunol 186, 2422-9.

3. Leonard, J.N., et al., 2008. The TLR3 signaling complex forms by cooperative receptor dimerization. Proc Natl Acad Sci U S A 105, 258-63.

4. Jiang, X., et al., 2008. Characterization of murine dendritic cell line JAWS II and primary bone marrow-derived dendritic cells in Chlamydia muridarum antigen presentation and induction of protective immunity. Infect Immun 76, 2392-401.

5. Naumann, K. et al., 2013. Activation of Dendritic cells by the novel Toll-like receptor 3 agonist Riboxxim® RGC100. Clin and Dev Immun, 2013.

F. RELATED PRODUCTS

product	art. no.
RIBOXXOL® (500 µg)	A-00102-0001
RIBOXXOL® (1 mg)	A-00102-0002
RIBOXXOL® negative control (500 µg)	A-00105-0001
RIBOXXOL® negative control (1 mg)	A-00105-0002
RIBOXXOL® red555 (100 µg)	A-00104-0001
RIBOXXOL® red555 (250 µg)	A-00104-0002
RIBOXXOL® CLINIgrade® (500 µg)	A-00101-0001
RIBOXXOL® CLINIgrade® (1 mg)	A-00101-0002

G. ORDER

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H. TECHNICAL SUPPORT

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