

RIBOXXOL® CLINlgrade®

RNA duplex - TLR3 ligand for vaccination studies

This datasheet (Version # 20160914) is valid for the following products:
Art.-No. A-00101-0001, Art.-No. A-00101-0002

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

A. PRODUCT INFORMATION

1. Content

Lyophilized RIBOXXOL (dry material).
Endotoxin-free RNase-DNase free water.
Endotoxin-free physiological water (NaCl 0.9%).

2. Storage

RIBOXXOL is shipped as lyophilized material at room temperature.
Short term storage: Dissolve in endotoxin-free physiological water (NaCl 0.9%) and store at 4°C for max. 3 months.
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 is a double-stranded RNA duplex consisting of cytosines, guanosines and inosines. It is prepared under germ-free conditions and is certified as endotoxin free (< 1 EU/mg, measurement by kinetic chromogenic LAL assay) and desalted material (at a concentration of 2 mg/ml, RIBOXXOL contains [Na⁺] < 5mM, [K⁺] < 1mM, [Cl⁻] < 5mM). The supplied physiological water (NaCl 0.9%) is certified endotoxin-free (< 0.25 EU/ml).

5. Chemical properties

CAS number: 63231-63-0
Ribonucleic acid duplex
Molecular weight: 34.5 KDa
Length: 50 bp
Base composition: Cytosines, inosines, guanosines
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 is a synthetic double stranded RNA (dsRNA). It has a length of 50 bp. It is composed of cytosines, inosines and guanosines.

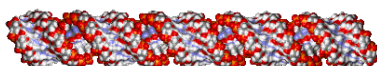


Fig. 1. Structural model of RIBOXXOL (RNA duplex, 50 bp).

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-1β, 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.

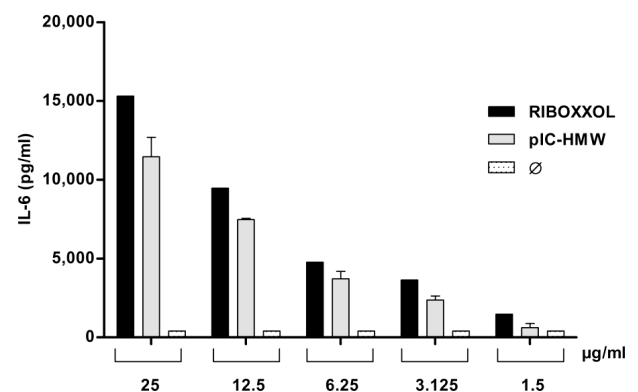


Fig. 2. Activation of human Dendritic Cells (DCs) by RIBOXXOL. DCs were isolated from a blood donor and incubated with RIBOXXOL at the indicated concentrations. IL-6 was measured in the supernatant after 48h. As a comparison, poly(I:C) High Molecular Weight (pIC-HMW) was used. The results shown correspond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

In animal models, RIBOXXOL enhances the adaptive innate response of peptide-based vaccines (in applications such as cancer, hepatitis), induces tumor specific T cell responses and high levels of cytokines (Th1). Moreover, RIBOXXOL displays a resistance to serum and body fluids (Fig. 3). It is therefore ideal for experimental set-up in cell culture supplemented with Fetal Calf Serum (FCS) and in small animal models.

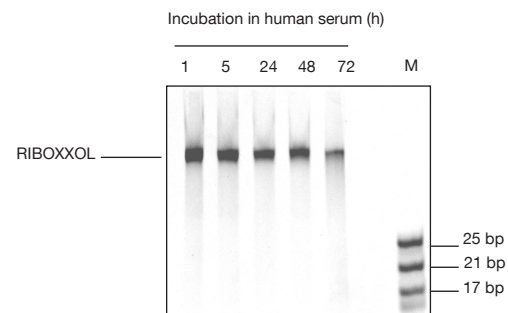


Fig. 3. Resistance of RIBOXXOL to serum degradation. RIBOXXOL (3 µM) was incubated with 80% human serum up to 72 h and analyzed on native PAGE after UV transillumination. M, RNA Marker.

C. FIELDS OF APPLICATION

RIBOXXOL CLINlgrade is dedicated to be used as

- Adjuvant for prophylactic vaccines (for bacterial or viral infections)
- Adjuvant for therapeutic vaccines (for tumor or viral diseases)
- For activation of cells implicated in adaptive and innate immunity in animal models.

D. METHODS

1. Preparation of RIBOXXOL CLINIgrade 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 vial.
- Add endotoxin-free physiological water (NaCl 0.9%) to the RIBOXXOL 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 CLINIgrade

Ligation of TLR3 by RIBOXXOL can be monitored using JAWS II dendritic cells. JAWs II DCs are murine immature myeloid dendritic cells. They have been 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.

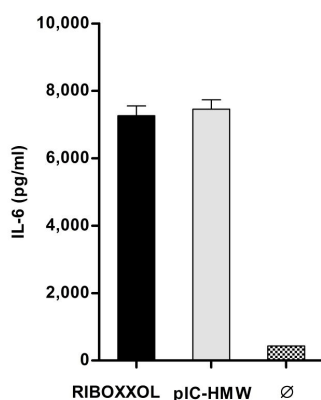


Fig. 4. Activation of JAWS II DCs by RIBOXXOL CLINIgrade. DCs were incubated with RIBOXXOL at 12.5 µg/ml. After 24h, IL-6 was measured in the supernatant. As a comparison, poly(I:C) High Molecular Weight (pIC-HMW) was used at the same concentration. The results shown correspond to the MEAN± SEM of 3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

3. Formulation of RIBOXXOL CLINIgrade for vaccines

RIBOXXOL CLINIgrade has been used in various studies as immune adjuvant for prophylactic or therapeutic vaccines. Formulation with peptides (up to 30-mers) as well as proteins (from 35 to 60 KDa) has shown that RIBOXXOL CLINIgrade is easy to formulate in solution.

Typically, dissolve RIBOXXOL CLINIgrade to ~ 2 mg/ml and mix the amount of antigen (peptide or protein) to the solution. Analyze the mix for presence of precipitates by visual control, and by measuring the OD₂₆₀ and the protein concentration (for example by BCA) in the supernatant. No precipitates would occur.

Store the mix at 4°C for 24 h. Measure the OD₂₆₀ and the protein concentration over 24 h. The concentration of RIBOXXOL CLINIgrade and protein in the solution should remain constant,

4. Dosis of RIBOXXOL CLINIgrade recommended for vaccines

RIBOXXOL CLINIgrade has been used in various studies as immune adjuvant for prophylactic or therapeutic vaccines. The usual recommended dosis for therapeutic vaccines experiments in small animals is 1-2 mg/m²/application for sub-cutaneous/ intra-dermal application. The usual recommended dosis for prophylactic vaccines experiments in small animals is 3-5 mg/kg/ application for intra-muscular application.

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 Riboxsim® 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® green488 (250 µg)	A-00103-0001
RIBOXXOL® green488 (500 µg)	A-00103-0002

G. ORDER

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H. TECHNICAL SUPPORT

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