LEXOGEN
The RNA Experts

RiBOCOP™

Select and Deplete

rRNA Depletion Kits for Fish

User Guide

Catalog Numbers:
241 (RiboCop rRNA Depletion Kit for Fish)

241UG837V0100

FOR RESEARCH USE ONLY. NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE.
INFORMATION IN THIS DOCUMENT IS SUBJECT TO CHANGE WITHOUT NOTICE.
Lexogen does not assume any responsibility for errors that may appear in this document.

PATENTS AND TRADEMARKS
RiboCop™ is a trademark of Lexogen. Lexogen is a registered trademark (EU, CH, US, CN, AU, NO, BR). RNasin® is a registered trademark of Promega Corporation. Bioanalyzer®, is a trademark of Agilent Technologies, Inc. Qubit™, RNaseZap™, RNAlater®, and ERCC are registered trademarks of Ambion, Inc. ThermoMixer® is a registered trademark of Eppendorf AG, DynaMag™ is a registered trademark of Thermo Fisher Scientific.

All other brands and names contained in this user guide are the property of their respective owners.

Lexogen does not assume responsibility for violations or patent infringements that may occur with the use of its products.

LIABILITY AND LIMITED USE LABEL LICENSE: RESEARCH USE ONLY
This document is proprietary to Lexogen. The RiboCop rRNA Depletion Kits are intended for use in research and development only. They need to be handled by qualified and experienced personnel to ensure safety and proper use. Lexogen does not assume liability for any damage caused by the improper use or the failure to read and explicitly follow this user guide. Furthermore, Lexogen does not assume warranty for merchantability or suitability of the product for a particular purpose.

The purchase of the product is subject to Lexogen general terms and conditions (www.lexogen.com/terms-and-conditions/) and does not convey the rights to resell, distribute, further sub-license, repackage, or modify the product or any of its components. This document and its content shall not be used or distributed for any other purpose and/or otherwise communicated, disclosed, or reproduced in any way without the prior written consent of Lexogen.

For information on purchasing additional rights or a license for use other than research, please contact Lexogen.

WARRANTY
Lexogen is committed to providing excellent products. Lexogen warrants that the product performs to the standards described in this user guide until the expiration date. Should this product fail to meet these standards due to any reason other than misuse, improper handling, or storage, Lexogen will replace the product free of charge or issue a credit for the purchase price. Lexogen does not provide any warranty if product components are replaced with substitutes.

Under no circumstances shall the liability of this warranty exceed the purchase price of this product.

LITERATURE CITATION
When describing a procedure for publication using this product, please refer to it as the RiboCop rRNA Depletion Kit for Fish.

We reserve the right to change, alter, or modify any product without notice to enhance its performance.

CONTACT INFORMATION

Lexogen GmbH
Campus Vienna Biocenter 5
1030 Vienna, Austria
www.lexogen.com
E-mail: support@lexogen.com

Support
E-mail: support@lexogen.com
Tel. +43 (0) 1 3451212-41
Fax. +43 (0) 1 3451212-99

Table of Contents

LEXOGEN

The RNA Experts

1. Overview

Lexogen's RiboCop rRNA Depletion Kits for Fish enable removal of ribosomal RNA (rRNA) from zebrafish (*Danio rerio*) and total RNA of other related fish species. RiboCop rRNA Depletion for Fish is perfectly suited for Next Generation Sequencing (NGS) and similar applications.

RiboCop uses a set of affinity probes for specific depletion of rRNA sequences. RiboCop probes efficiently remove ribosomal RNA and therefore afford a comprehensive view of transcriptome composition. Samples void of 28S, 18S, 5.8S, 45S, 5S, mt16S, and mt12S ribosomal sequences are obtained within 1.5 hours of total processing time. No enzymatic reactions or mechanical shearing steps are involved, leaving full-length transcripts intact for downstream processing.

Affinity probes are mixed with total RNA and denatured, facilitating access of probes to target sequences. Afterwards, hybridization is performed at elevated temperature. Depletion beads are washed and used to remove probes that are hybridized to ribosomal RNA from the solution. A final purification step using magnetic beads rounds off the procedure. Recovered RNA may be directly used for NGS library preparation, with sufficient volume for optional quality control. The entire protocol is automation-friendly, using magnetic beads for depletion and purification purposes.

The recommended input range for RiboCop is 1 - 1,000 ng of total RNA. RiboCop is suitable for intact or degraded RNA (Appendix A, p.12). Results and depletion rates can vary depending on the species, RNA input amount, and RNA integrity.

RiboCop is recommended for downstream NGS library preparation using the CORALL RNA-Seq V2 Library Prep Kit (Cat. No. 171 - 176). In addition, RiboCop is also compatible with RNA-Seq library preps from other vendors.

RiboCop rRNA Depletion Kits are fully compatible with each other. RiboCop for Bacteria Kits (Cat. No. 125 - 127) can be used together with RiboCop for Fish Kits to co-deplete bacterial and host rRNA. Please contact support@lexogen.com for more information.

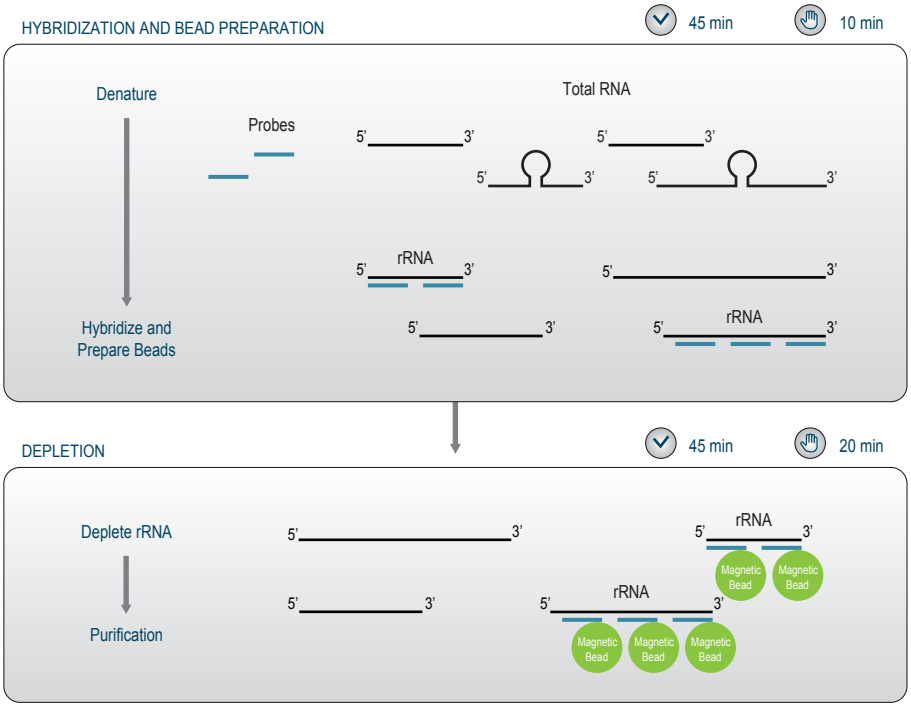

Figure 1. Schematic overview of the RiboCop workflow.

2. Kit Components and Storage Conditions

Kit Component	Tube Label	Volume* for		Storage
		24 preps	96 preps	
Hybridization Solution	**HS** ●	106 µl	423 µl	+4 °C
Probe Mix FISH	**FISH** ●	132 µl	528 µl	+4 °C / -20 °C
Depletion Beads	**DB** ●	1,980 µl	7,920 µl	+4 °C
Depletion Solution	**DS** ○	4,752 µl	19,008 µl	+4 °C
Purification Beads	**PB** ○	634 µl	2,535 µl	+4 °C
Purification Solution	**PS** ○	2,852 µl	11,405 µl	+4 °C
Elution Buffer	**EB** ○	317 µl	1,268 µl	+4 °C / -20 °C

*including ≥10 % surplus

Upon receiving the RiboCop kit, store all components at +4 °C. **EB** ○ and Probe Mix **FISH** ● can also be stored at -20 °C but avoid frequent freeze-thaw cycles. Before use, check the contents of **PS** ○ which may precipitate during shipping. If a precipitate is visible or the content appears turbid, incubate at 37 °C until solution components dissolve completely. Equilibrate to room temperature again before use.

3. User-Supplied Consumables and Equipment

Check to ensure that you have all of the necessary materials and equipment before beginning rRNA depletion. All reagents, equipment, and labware must be free of nucleases and nucleic acid contamination.

ATTENTION: Before starting this protocol, please read the General Guidelines for Lexogen Kits, which is available online. This document provides a detailed overview of RNA and kit component handling, as well as general RNA input requirements.

It is also strongly recommended to carefully read the input guidelines and notes listed on Appendix A, p.12.

Reagents

- 80 % fresh ethanol (washing of Purification Beads, **PB** ○).

Equipment

- Magnetic rack or plate e.g., for 1.5 ml tubes: BcMag Magnetic separator-24, article# MS-03 from Bioclone; for 96-well plates: 96S Super Magnet Plate, article# A001322 from Alpaqua, or Dynamag-96 Side/Side Skirted. Magnets, article # 12331D/12027 from Thermo Fisher.
- Benchtop centrifuge for spinning down liquids.
- Vortex mixer.
- Calibrated single-channel pipettes for handling 1 µl to 1,000 µl volumes.
- Thermomixer.
- UV-spectrophotometer to quantify RNA.

Labware

- Suitable low-binding pipette tips (pipette tips with aerosol barriers recommended).
- 1.5 ml tubes, low binding, certified ribonuclease-free.
- 200 µl PCR tubes or 96-well plates and caps or sealing foil.
- Benchtop cooler or ice pellets in ice box (for short-term storage of RNA).

Optional Equipment and Solutions

- Automated microfluidic electrophoresis station (Agilent Technologies 2100 Bioanalyzer).
- Benchtop fluorometer and appropriate assays (for RNA quality control and quantification).
- Agarose gels, dyes, and electrophoresis rig (for RNA quality control).
- RNaseZap.
- RNase inhibitor.

The complete set of materials, reagents, and labware for quality control is not listed. Consult Appendix A, p.12 for more information.

4. Detailed Protocol RiboCop for Fish

Preparation

Hybridization	Bead Preparation and Depletion	Purification
HS ● } equilibrated **FISH** ● } at RT	**DB** ● } equilibrated **DS** ○ } at RT	**PB** ○ – stored at +4 °C **PS** ○ – stored at +4 °C **80 % EtOH** – provided by user, **prepare fresh**! **EB** ○ – thawed at RT or stored at +4 °C
Thermomixer set to 75 °C / 1,250 rpm	Thermomixer set to 60 °C / 1,250 rpm	Equilibrate all reagents to room temperature for 30 minutes prior to use

Hybridization

The depletion Probe Mix for Fish (**FISH** ●) and total RNA are mixed, denatured, and hybridized.

ATTENTION: Important notes for RiboCop hybridization steps:

- If a thermomixer is not available, input amounts ≤500 ng of total RNA can be processed in PCR tubes or plates and incubated in a thermocycler, without shaking.
- Bead Washing (p.9) can be performed during the incubation at step 5.
- Place the Purification Beads (**PB** ○) and Purification Solution (**PS** ○) for step 16 at room temperature prior to starting the protocol, to ensure these have at least 30 minutes to equilibrate.

1	Prepare 1 - 1,000 ng of total RNA in a total volume of 26 µl. Dilute using RNase-free water if required.
2	Add 4 µl Hybridization Solution (**HS** ●).
3	Add 5 µl Probe Mix (**FISH** ●) and mix thoroughly until homogeneous.
4	Denature samples using a thermomixer at 75 °C for 5 minutes with agitation at 1,250 rpm.
5	Decrease the temperature of the thermomixer to 60 °C and incubate the samples for 30 minutes with agitation at 1,250 rpm. **OPTIONAL:** Transfer the samples from step 4 to a second thermomixer set to 60 °C.

Bead Washing

Depletion Beads (**DB** ●) must be washed before use. These steps may be performed as a batch for up to 6 samples.

ATTENTION: Mix Depletion Beads (**DB** ●) fully before use.

6	Transfer 75 µl Depletion Beads (**DB** ●) per reaction to a fresh tube.
7	Place the tube onto the magnet and let the beads collect for 2 - 5 minutes or until the supernatant is completely clear. Remove and discard the supernatant with a pipette while the tube remains in contact with the magnet.
8	Add 75 µl Depletion Solution (**DS** ○) per reaction to the beads. Remove the tube from the magnet and resuspend the beads.
9	Place the tube back onto the magnet and let the beads collect for 2 - 5 minutes or until the supernatant is completely clear. Remove and discard the supernatant.
10	Repeat this washing step once (for a total of two washes).
11	Resuspend the beads in 30 µl Depletion Solution (**DS** ○) per reaction.

Depletion

Depletion Beads (**DB** ●) are used to remove the probe-hybridized ribosomal RNA.

ATTENTION: Avoid the formation of air bubbles while mixing.

12	Spin down the hybridized sample from step 5 and add 30 µl of freshly prepared beads from step 11. Mix by pipetting up and down at least 8 times, or until homogeneous.
13	Put the sample back to the thermomixer and incubate at 60 °C for 15 minutes with agitation at 1,250 rpm.
14	Spin down briefly then place the sample on the magnet and let the beads collect for 5 minutes.
15	Recover and transfer 60 µl of the supernatant containing the rRNA-depleted RNA to a fresh tube. Avoid disturbing the collected beads to prevent rRNA contamination. **ATTENTION:** Do not throw away the supernatant.

Purification

The depleted RNA is purified using magnetic beads. The purification reagents (**PB** ○, **PS** ○, and **EB** ○) should equilibrate for 30 minutes at room temperature before use. The Purification Beads (**PB** ○) must be fully resuspended before use. Thorough mixing by pipetting or vortexing is recommended.

16	Add 24 µl Purification Beads (**PB** ○) and 108 µl Purification Solution (**PS** ○) to the supernatant. Mix well by pipetting. Incubate for 5 minutes at room temperature.
17	Place the sample onto a magnet and let the beads collect for 5 - 10 minutes or until the supernatant is completely clear. **NOTE:** The speed of bead collection depends on the strength of the magnet.
18	Remove and discard the clear supernatant without removing the sample from the magnet. Make sure that accumulated beads are not disturbed.
19	Add 120 µl of 80 % EtOH and incubate the beads for 30 seconds. Leave the sample on the magnet as beads should not be resuspended during this washing step. Remove and discard the supernatant. **ATTENTION:** When using 1.5 ml tubes use 150 µl of 80 % EtOH to ensure beads are fully covered by EtOH.
20	Repeat this washing step once for a total of two washes. Make sure the supernatant is removed completely.
21	Leave the sample in contact with the magnet and let the beads dry for 5 -10 minutes or until all ethanol has evaporated. **ATTENTION:** Dry the beads at room temperature only and do not let the beads dry too long (visible cracks appear). This will negatively influence the elution and the resulting RNA recovery.
22	Add 12 µl of Elution Buffer (**EB** ○), remove the sample from the magnet, and resuspend the beads properly in **EB** ○. Incubate for 2 minutes at room temperature.
23	Place the sample onto the magnet and let the beads collect for 2 - 5 minutes or until the supernatant is completely clear.
24	Transfer 10 µl of the supernatant into a fresh tube. Depleted RNA is now ready for quality control and downstream use (e.g., for NGS library preparation). ☞ Safe stopping point. RNA can be stored at ≤-20 °C at this point.

5. Short Procedure RiboCop for Fish

ATTENTION: Spin down solutions before opening tubes or plates!

45 min Hybridization and Bead Washing

	Hybridization
☐	Prepare 1 - 1,000 ng total RNA in 26 µl.
☐	Add 4 µl **HS** ●.
☐	Add 5 µl **FISH** ● and mix until homogeneous.
☐	Denature for 5 min at 75 °C / 1,250 rpm.
☐	Incubate for 30 min at 60 °C / 1,250 rpm.
	Bead Washing
☐	Resuspend **DB** ●, transfer 75 µl to a fresh tube.
☐	Place on magnet for 2 - 5 min, discard supernatant.
☐☐	Resuspend beads in 75 µl **DS** ○, incubate 2 min on magnet, discard supernatant. Repeat once.
☐	Resuspend beads in 30 µl **DS** ○.

45 min Depletion and Purification

	Depletion
☐	Spin down hybridized sample. Add 30 µl of prepared beads. Mix by pipetting 8x or until homogeneous.
☐	Incubate for 15 min at 60 °C / 1,250 rpm. Spin down.
☐	Place on magnet for 5 min.
☐	Transfer 60 µl supernatant to a fresh tube. **ATTENTION:** The supernatant contains the rRNA depleted RNA.
	Purification
☐	Add 24 µl **PB** ○ and 108 µl **PS** ○, mix well, incubate for 5 min at RT.
☐	Place on magnet for 5 - 10 min, discard supernatant.
☐☐	Wash the beads twice with 120 - 150 µl 80 % EtOH, 30 sec. **ATTENTION:** Use 150 µl for 1.5 ml tubes.
☐	Air dry beads for 5 - 10 min. **ATTENTION:** do not over dry the beads!
☐	Add 12 µl **EB** ○, remove from magnet, mix well, incubate 2 min at RT.
☐	Place on magnet for 2 - 5 min, transfer 10 µl of the supernatant to a fresh tube. ☞ Safe stopping point.

6. Appendix A: RNA Requirements and Results

Potential Contaminants

RNA samples should be free of salts, metal ions, and organic solvents, which can be carried over from RNA extraction. Several sources of contamination (e.g., proteins, chaotropic salts, and phenol) can be detected with a UV-Vis spectrophotometer. An acceptably pure RNA sample should have an A260/A280 ratio between 1.8 and 2.1. The A260/A230 ratio should also be greater than 1.8. Contaminants may have a negative impact on the efficiency of the protocol. For isolation of high quality total RNA we recommend Lexogen's SPLIT RNA Extraction Kit (Cat. No. 008).

RNA Input Amount and Quality

The recommended input range for RiboCop is 1 - 1,000 ng of total RNA. RiboCop is suitable for intact or degraded RNA. Results and depletion rates can vary depending on the species, RNA input amount, and RNA integrity.

Spike-in controls, including Lexogen's Spike-In RNA Variant Controls (SIRVs, Cat. No. 025, 050, 051, and 141), should be added to the RNA sample before RiboCop (or prior to DNase treatment). The spike-in amount must be calculated relative to the target RNA fraction (e.g., ribo-depleted RNA). We recommend targeting ~1 % of SIRV-mapped reads for higher quality samples (RIN >7). For assistance with SIRV spike-in calculation, please contact support@lexogen.com.

Typical Results

Quality control of depleted RNA (using microcapillary electrophoresis, e.g., Bioanalyzer PicoChip, Agilent Technologies) is recommended prior to conducting library preparation and sequencing runs. Successful rRNA depletion results in removal of 28S and 18S rRNA peaks. These rRNA peaks usually dominate electropherograms of intact total RNA and should be absent after RiboCop depletion as shown in Figure 2.

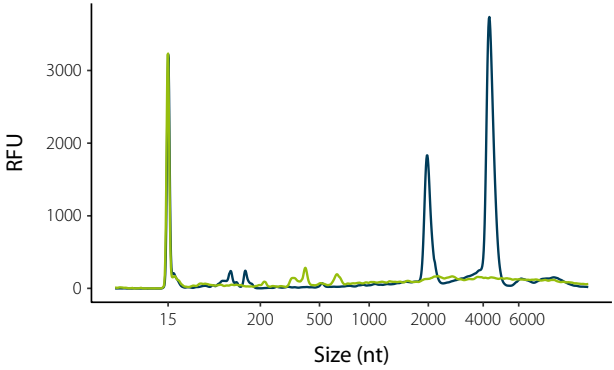

Figure 2. Results of RiboCop Fish ribo-depletion. 1 µg Zebrafish RNA was used as input for RiboCop Fish (green trace) and compared to 5 ng undepleted *Danio rerio* RNA (blue trace) showing 28S and 18S rRNA peaks.

Simultaenous Depletion of Host and Bacterial rRNA

Lexogen offers a Suggested Protocol for simultaneous host and bacterial rRNA depletion. For more information, please contact support@lexogen.com.

7. Appendix B: Downstream Processing

This protocol was tested extensively with *Danio rerio* total RNA. Depleted RNA samples are ideally suited for downstream NGS library preparation using Lexogen's CORALL RNA-Seq V2 Library Prep Kits (Cat. No. 171 - 176). Up to 10 µl of the eluted rRNA-depleted RNA may be used as input for CORALL library preparation.

8. Appendix C: Revision History

Publication No. / Revision Date	Change	Page
241UG837V0100 Aug. 14, 2024	Initial Release.	

LEXOGEN
The RNA Experts

Associated Products:

008 (SPLIT RNA Extraction Kit)
022 (Purification Module with Magnetic Beads)
025, 050, 051, 141 (SIRVs Spike-in RNA Variant Control Mixes)
125-127 (RiboCop rRNA Depletion Kits for Bacteria)
144 (RiboCop rRNA Depletion Kits for Human/Mouse/Rat)
145 (RiboCop rRNA Depletion Kits for Human/Mouse/Rat Plus Globin)
171-176 (CORALL RNA-Seq V2 Library Prep Kits)
190 (RiboCop rRNA Depletion Kits for Yeast)
237 (RiboCop rRNA Depletion Kits for Plants)

RiboCop rRNA Depletion Kit for Fish · User Guide

Lexogen GmbH
Campus Vienna Biocenter 5
1030 Vienna, Austria
Telephone: +43 (0) 1 345 1212-41
Fax: +43 (0) 1 345 1212-99
E-mail: support@lexogen.com

Lexogen, Inc.
51 Autumn Pond Park
Greenland, NH 03840, USA
Telephone: +1-603-431-4300
Fax: +1-603-431-4333
www.lexogen.com