

The background of the entire page is decorated with a series of translucent blue spheres of various sizes, some of which are connected by thin, light blue lines, creating a molecular or network-like structure.

LEXOGEN

The RNA Experts

RiBO COP™

Select and Deplete

rRNA Depletion Kits for Bacteria User Guide

Catalog Numbers:

125 (RiboCop rRNA Depletion Kit for Mixed Bacterial Samples (META))

126 (RiboCop rRNA Depletion Kit for Gram Negative Bacteria (G-))

127 (RiboCop rRNA Depletion Kit for Gram Positive Bacteria (G+))

125UG246V0102

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 registered trademark of Agilent Technologies, Inc. Qubit™ is a trademark of Thermo Fisher Scientific. RNaseZap™ is a trademark 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 right to resell, distribute, further sublicense, repackage, or modify the product or any of its components. This document and its contents 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 Bacteria.

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

1. Overview.	4
2. Kit Components and Storage Conditions	6
3. User-Supplied Consumables and Equipment.	7
4. Detailed Protocol RiboCop for Bacteria	8
5. Short Procedure RiboCop for Bacteria.	12
6. Appendix A: RNA Requirements and Results.	13
7. Appendix B: Downstream Processing	14
8. Appendix C: Revision History.	15

1. Overview

Lexogen's RiboCop rRNA Depletion Kits for Bacteria enable removal of ribosomal RNA (rRNA) from bacterial total RNA and are suited for Next Generation Sequencing (NGS) and similar applications.

RiboCop uses a set of affinity probes for specific depletion of rRNA sequences. The number and positioning of probes are designed for compatibility with intact and fragmented input RNA. RiboCop probes efficiently remove ribosomal RNA and therefore afford a comprehensive view of transcriptome composition. Samples void of 23S, 16S, and 5S 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 rRNA 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 efficient depletion with RiboCop rRNA Depletion Kits for Bacteria is 1 - 1,000 ng of total RNA for monocultures of Gram negative or Gram positive bacteria using the respective G- and G+ Probe Mixes (Cat. No. 126 and 127, respectively). The META Probe Mix (Cat. No. 125) is primarily suited for depletion of 1 - 1,000 ng of total RNA from mixed bacterial samples (e.g., for meta-transcriptome analysis of complex communities or microbiome samples). In this case, depletion rates may vary depending on the complexity and species composition within the population subjected to depletion. The META Probe Mix may also be used for depletion of 1 - 100 ng of RNA obtained from monocultures. RiboCop is suitable for intact or degraded RNA, including FFPE samples (Appendix A, p.13).

RiboCop is recommended for downstream NGS library preparation using the CORALL RNA-Seq V2 Library Prep Kit (Cat. No. 171 - 176). In addition, previous versions of CORALL RNA-Seq Library Prep Kits can also be used for downstream NGS library preparation.

RiboCop rRNA Depletion Kits for Bacteria (Cat. No. 125 - 127) are also fully compatible with RiboCop Kits for Human/Mouse/Rat (Cat. No. 144-145) to co-deplete bacterial and host rRNA. Please contact support@lexogen.com for more information.

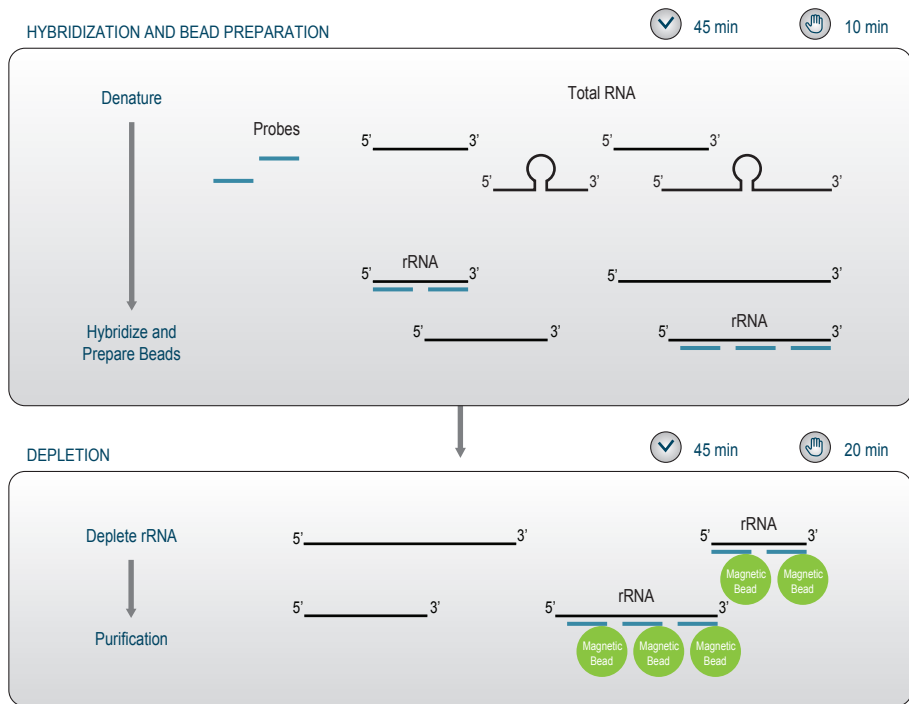


Figure 1. Schematic overview of the RiboCop workflow.

2. Kit Components and Storage Conditions

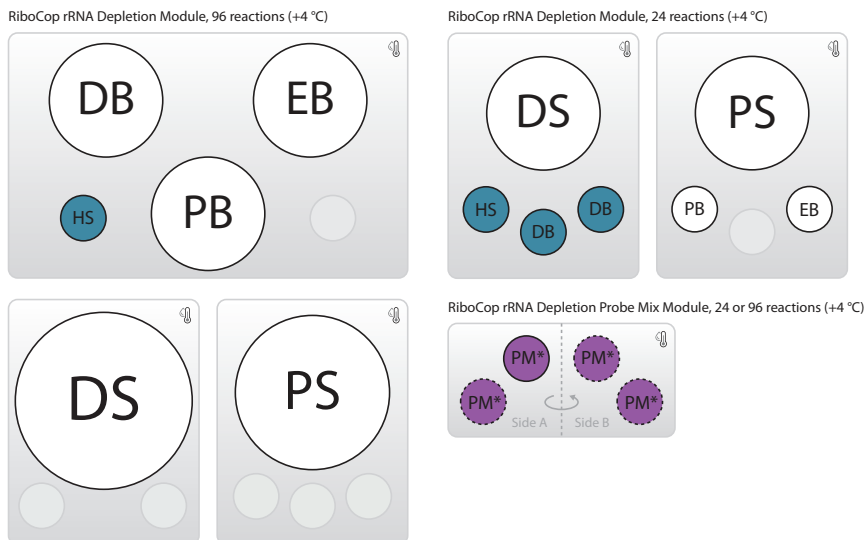


Figure 2. Location of kit components. Each RiboCop rRNA Depletion Kit for Bacteria consists of a Depletion Module and one of the three available Probe Mix Modules. RiboCop rRNA Depletion for Bacteria Kits are available for Mixed Bacterial Samples (META, Cat. No. 125.24) and for Gram Negative (G-, Cat. No. 126.24) or Gram Positive Bacteria (G+, Cat. No. 127.24). 96 prep kits contain 4 tubes of the respective Probe Mix (PM* = META, G-, or G+).

Kit Component	Tube Label	Volume* for		Storage
		24 preps	96 preps	
Hybridization Solution	HS ●	106 µl	423 µl	🔒 +4 °C
Probe Mixes META, G-, or G+	META / G- / G+ ●	132 µl	4 x 132 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 Mixes (**META, G-, G+**) ● can also be stored at -20 °C but frequent freeze-thaw cycles should be avoided. 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.13.

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.13 for more information.

4. Detailed Protocol RiboCop for Bacteria

Probe Mix Selection

The following Probe Mixes are available: META, G-, and G+. The G- and G+ Probe Mixes are specifically designed for efficient depletion of monocultures of Gram positive and Gram negative bacteria up to 1,000 ng total RNA input. The META Probe Mix is primarily suited for depletion of 1 - 1,000 ng of total RNA from mixed bacterial samples (e.g., for metatranscriptome analysis of complex communities or microbiome samples). In this case, depletion rates may vary depending on the complexity and species composition within the population subjected to depletion. The META Probe Mix may also be used at 1 - 100 ng when working with monocultures. It is recommended to use total RNA input amounts compatible to the library preparation kit used for library generation after rRNA depletion. Typically, 100 ng total RNA input is recommended for initial experiments.

Input type	Probe Mix	Input recommendations
Mixed Bacterial Samples	META	1 - 1,000 ng for mixed communities 1 - 100 ng for G- / G+ monocultures
Gram Negative Bacteria	G-	1 - 1,000 ng for Gram Negative monocultures
Gram Positive Bacteria	G+	1 - 1,000 ng for Gram Positive monocultures

Preparation

Hybridization	Bead Washing and Depletion	Purification
HS ● } equilibrated PM ● } 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 (**META**, **G-**, or **G+** ●) and total RNA are mixed, denatured, and hybridized.

ATTENTION: Important notes for RiboCop hybridization steps:

- If a thermomixer is not available, input amounts ≤ 250 ng of total RNA can be processed in PCR tubes or plates and incubated in a thermocycler, without shaking.
- Bead Washing 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 μ l. Dilute using RNase-free water if required. **ATTENTION:** If using the META kit with a monoculture, limit the total RNA input to 100 ng.
- 2 Add 4 μ l Hybridization Solution (**HS** ●).
- 3 Add 5 μ l Probe Mix (**META**, **G-**, or **G+** ●) 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 μ l 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 μ l 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 washed 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 Bacteria

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. **ATTENTION:** Use 1 - 100 ng total RNA when using the META Probe Mix for monocultures.
- ☐ Add 4 µl **HS** ●.
- ☐ Add 5 µl Probe Mix (**META**, **G-**, or **G+**) ●, 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 washed 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.

RNA Input Amount and Quality

The recommended input range for RiboCop is 1 - 1,000 ng of total RNA for monocultures using the G+ and G- Probe Mixes, and 1-100 ng for treating monocultures with the META Probe Mix. In addition, the META Probe Mix is suitable for depletion of complex, mixed community samples from 1 - 1,000 ng of total RNA. It is recommended to use 100 ng of total RNA for initial trials. RiboCop is suitable for intact or degraded RNA, including Formalin Fixed Paraffin-Embedded (FFPE) samples. Spike-in controls, including Lexogen's Spike-In RNA Variant Controls (SIRVs, Cat. No. 025, 050, 051, 141), should be added to the RNA sample before RiboCop (or prior to DNase treatment for bacterial and/or FFPE samples). The spike-in amount must be calculated relative to the target RNA fraction (e.g., ribo-depleted RNA). We recommend targeting 0.1 - 0.2 % of SIRV-mapped reads for FFPE samples, or 1 % for higher quality samples. For assistance with SIRV spike-in calculation, please contact support@lexogen.com.

Simultaneous 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 *E. coli*, *P. aeruginosa*, and *B. subtilis* total RNA and complex microbiome total RNA isolated from stool samples. 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). In addition, previous versions of CORALL RNA-Seq Library Prep Kits can also be used for downstream NGS library preparation. Up to 10 µl of the eluted ribo-depleted RNA may be used as input for CORALL library preparation. RNA treated with RiboCop for Bacteria is compatible with all random-primed total RNA library prep kits and adapter ligation protocols.

8. Appendix C: Revision History

Publication No. / Revision Date	Change	Page
125UG246V0102 Jul. 14, 2022	Updated Legal Disclaimer text.	2
	Updated Kit Components Figure 2 and Table to reflect current packaging and storage requirements.	6
	Added link to General Guidelines.	7
	Removed General section.	8
	Included reference to simultaneous host and bacterial rRNA depletion.	13
125UG246V0101 Aug. 31, 2020	Added Attention note to step 1.	11, 14
	Minor text edits.	4, 10
125UG246V0100 Feb. 17, 2020	Initial Release.	

Associated Products:

022 (Purification Module with Magnetic Beads)
025, 050, 051, 141 (SIRVs Spike-in RNA Variant Control Mixes)
095 (CORALL Total RNA-Seq Library Prep Kit)
117 - 119, 132 - 134 (CORALL Total RNA-Seq Library Prep Kit with UDIs)
144 (RiboCop rRNA Depletion Kit for Human/Mouse/Rat (HMR) V2)
171-176 (CORALL RNA-Seq V2 Library Prep Kits)
183-184 (CORALL RNA-Seq V2 Library Prep Kit with RiboCop (HMR))
185-186 (CORALL RNA-Seq V2 Library Prep Kit with RiboCop (HMR+Globin))

RiboCop rRNA Depletion Kits for Bacteria · 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 GmbH, 2022

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