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The RNA Experts

RiBOCOP™

Select and Deplete

rRNA Depletion Kit for Plants

User Guide

Catalog Numbers:
237(RiboCop rRNA Depletion Kit for Plants)

237UG789V0100

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1. Overview

Lexogen's RiboCop rRNA Depletion Kit for Plants enables removal of ribosomal RNA (rRNA) from plant total RNA and is 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 rRNA and afford a comprehensive view of transcriptome composition. Samples void of cytoplasmic (5.8S, 18S and 25S), mitochondrial (5S, 18S and 26S) and chloroplast (4.5S, 5S, 16S and 23S) ribosomal sequences are obtained within 1.5 hours of total processing time. No enzymatic reactions or mechanical shearing steps are involved, leaving full-length tran-scripts 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 for Plant is 1 - 1,000 ng of total RNA. RiboCop is suitable for intact or degraded RNA with a minimum recommended RNA input of 10 ng (Appendix A, p.12).

The RiboCop for Plant Probe Mix has been optimized for increased depletion efficiency even with difficult samples. Probes are designed to eliminate off-target effects. Thus, RiboCop maintains consistent transcript expression after depletion.

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

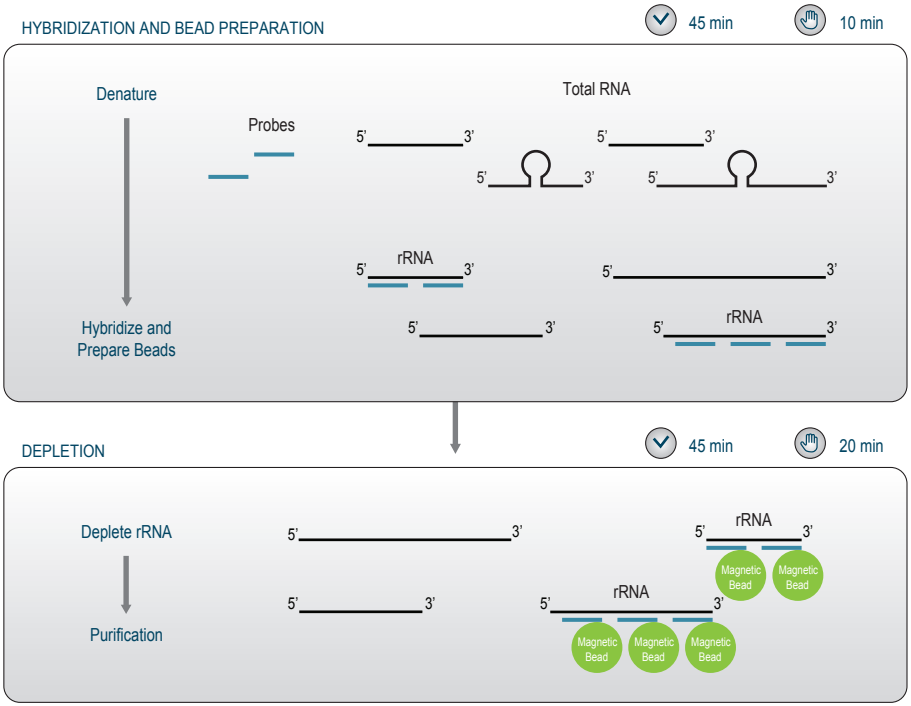

Figure 1. Schematic overview of the RiboCop workflow.

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
Plant Probe Mix	**PPM** ●	132 µl	4x 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 Mix (**PPM**) ● 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 p.12.

Reagents

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

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 Plants

Preparation

Hybridization	Bead Preparation and Depletion	Purification
HS ● } equilibrated **PPM** ● } 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 95 °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 Plants (**PPM** ●) and total RNA are mixed, denatured, and hybridized.

ATTENTION: Important notes for RiboCop hybridization steps:

- RiboCop for Plants recommend input amounts are 1 - 1000 ng intact total RNA, for degraded RNA a minimum of 10 ng is recommended.
- 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 Plant Probe Mix (**PPM** ●) and mix thoroughly until homogeneous.
4	Denature samples using a thermomixer at 95 °C for 2 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 Plants

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 **PPM** ● and mix until homogeneous.
☐	Denature for 2 min at 95 °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 for 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 for Plants is 1 - 1,000 ng of total RNA. RiboCop is suitable for intact or degraded RNA. For degraded RNA, a minimum of 10 ng RNA input is recommended for efficient depletion. 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 the non-rRNA content of a samples. For assistance with SIRV spike-in calculation, please contact support@lexogen.com.

7. Appendix B: Downstream Processing

This protocol was tested extensively with intact and degraded total RNA obtained from *A. thaliana*. 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
237UG789V0100 Apr. 24, 2024	Initial Release.	

9. Appendix D: Notes

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Associated Products:

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)
171-176 (CORALL RNA-Seq V2 Library Prep Kits)

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