Detailed protocol

riboLASSO

Ultra-specific rRNA depletion for RNA-Seq

For Research Use Only: Not for use in human or animal therapeutic or diagnostic procedures.

Purpose of the kit

Ribosomal RNA (rRNA) can constitute a large fraction of total RNA and can therefore consume a substantial proportion (80-90%) of sequencing reads. Depleting rRNA before sequencing increases the fraction of reads available for RNAs of interest and can improve the efficiency of total RNA-Seq workflows.

riboLASSO is designed to remove rRNA from total RNA before downstream library preparation and sequencing. The standard workflow uses a 100 µL reaction and consists of reaction setup, annealing, centrifugation, and recovery of the rRNA-depleted RNA-containing supernatant.

Product Description

riboLASSO is an ultra-specific rRNA depletion solution from total RNA. The core depletion procedure can be completed in less than 60 minutes, excluding optional DNase I treatment, RNA purification, concentration, and downstream library preparation.

The protocol is intended as a generic workflow. Use the riboLASSO product version corresponding to the target organism or sample type specified on the product label. The same core procedure applies across supported riboLASSO versions unless product-specific instructions state otherwise.

riboLASSO can remove more than 95% of rRNA, with consistent performance across replicate samples. Performance can vary with sample type, RNA quality, target organism, sample preparation, and downstream analytical workflow

Materials

Materials included in the package

	24 reactions	**48 reactions**	**96 reactions**
riboLASSO Component B	1 × 500 µL	1 × 1 mL	2 × 1 mL
riboLASSO Component X	1 × 500 µL	1 × 1 mL	2 × 1 mL
Nuclease-free water	1 × 1 mL	1 × 1 mL	1 × 1 mL

Shipping, storage & shelf-life

- The riboLASSO kit is shipped refrigerated at approximately 4 °C.
- riboLASSO Component B: store at 4 °C. Stable for at least 6 months under the recommended storage condition. Freezing is not recommended.
- riboLASSO Component X: store at 4 °C for routine use. Stable for at least 6 months. For longer-term storage, Component X may be stored at −20 °C.
- Recovered rRNA-depleted RNA: if not processed immediately, store at −80 °C.

Additional equipment and materials required

- 200 µL RNase-free PCR tubes or compatible PCR plates.
- RNase-free, preferably low-retention pipette tips and tubes.
- Calibrated pipettes suitable for 1–100 µL volumes.
- Vortex mixer.
- Microcentrifuge or plate centrifuge capable of the required relative centrifugal force.
- Thermocycler
- Ice bucket or cold block for RNA samples before reaction setup.

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Optional:

- RNase-free DNase I; use according to the manufacturer's instructions.
- RNase inhibitor; recommended for sensitive RNA samples and used according to the manufacturer's instructions.
- RNA purification or cleanup reagents; selected according to downstream workflow and required RNA concentration.
- Centrifuge tube adaptors for 200 µL. These are not supplied by default; 3D-printed adaptors may be requested when placing an order if required for the user's centrifuge setup.

Protocol

General remarks and considerations

- Use standard RNase-free technique throughout the workflow. Keep the work area clean, wear gloves, use RNase-free tubes and tips, and minimize the time RNA-containing tubes remain open.
- High-quality total RNA is recommended. The total RNA input range is 1 ng–1 µg per 100 µL reaction.
- The reaction volume can be scaled to greater or smaller volumes than 100 µL. Ensure proper heat distribution in thermal cycler when using larger volumes.
- The reagent temperature during setup is not critical; riboLASSO components may be used directly from 4 °C or after equilibration to room temperature.
- The protocol can be performed in 200 µL PCR tubes or compatible plate formats. Throughput is determined by the user's thermal cycler and centrifuge configuration.

Preparation

1. Keep RNA samples on ice before reaction setup. Minimize freeze-thaw cycles.
2. Confirm that the RNA amount per reaction is between 1 ng and 1 µg and that the sample volume does not exceed 60 µL.
3. Ensure the thermal cycler is available and can run the annealing program described later.
4. Ensure a suitable centrifuge is available before beginning the reaction so that samples can be processed promptly after annealing.
5. Do not exceed 1 µg total RNA or 60 µL RNA sample volume in the standard 100 µL reaction.

Assembly

6. Set up each reaction in a 200 µL RNase-free PCR tube or compatible plate well. Add the components in the order shown below.

Component	Volume per 100 µL reaction	Final concentration
riboLASSO Component B (5X)	20 µL	1X
riboLASSO Component X (5X)	20 µL	1X
Total RNA	1 ng–1 µg in up to 60 µL	1 – 1000 ng
Nuclease-free water	to 100 µL	

7. Quickly vortex the assembled reaction to mix.
8. Briefly spin down to collect liquid from the tube walls and cap, or briefly centrifuge the plate if using a plate format.

Note: For sensitive RNA samples, an RNase inhibitor may be included in the reaction mixture. Use according to the manufacturer's instructions.

Annealing

9. Place the mixed reaction in a thermal cycler and run the following annealing program:

Temperature	Time / transition
95 °C	3 minutes
95 °C → 80 °C	Quick cooling
80 °C → 20 °C	20 minutes
20 °C	Hold until centrifugation

10. Centrifuge the annealed samples at >10,000 × g for 30 minutes.
11. After centrifugation, a clearly visible colored pellet should be present.

Note: Centrifugation may be performed from 4 °C to room temperature.
Note: The standard condition remains >10,000 × g for 30 minutes. Lower centrifugal forces >2,700 × g, may also be used when required by compatible plate formats or centrifuge limitations. The required centrifugation time should be optimized by the user.
Optional accessory: Centrifuge tube adaptors are not supplied by default. If required for a specific centrifuge setup, 3D-printed adaptors may be requested when placing the order.

Recovery of rRNA-depleted RNA sample

12. Using an RNase-free pipette tip, aspirate the supernatant from the top of the liquid, keeping the pipette tip away from the pellet.
13. Transfer up to 70 µL of supernatant to a fresh RNase-free tube or destination plate well. This is the recommended safe recovery volume.
14. The recovered supernatant is the rRNA-depleted RNA fraction. Discard the pellet after the desired supernatant has been recovered.
15. Proceed directly to RNA purification or store the recovered rRNA-depleted RNA at −80 °C if it will not be used immediately. The RNA samples must be purified before sequencing library preparation to remove salts and buffer components.

Important: Do not disturb or aspirate the pellet. Pellet carryover can contaminate the recovered fraction.
Note: If the pellet can be avoided confidently, up to 80 µL of supernatant may be recovered.
Note: The final rRNA-depleted sample contains approximately 5 mM Tris, 1 mM EDTA, and 150 mM NaCl.

(**Optional**) DNAse I treatment

The recovered rRNA-depleted RNA can be used for library preparation without DNase I treatment if compatible with the downstream workflow. Sequencing has been performed successfully without a DNase I step.

1. If removal of residual DNA is required, treat the recovered rRNA-depleted RNA with an RNase-free DNase I.
2. Perform the treatment according to the DNase I manufacturer's instructions.
3. Use a standard RNA purification or cleanup procedure appropriate for the downstream application, buffer requirements, and target RNA concentration.

Snapshot Protocol