siTOOLs BIOTECH

Efficient and rapid rRNA-depletion solution for total RNA

High-Input riboPOOL™ Kit: User Guide

For Research Use Only.

riboPOOL™ Kit: User Guide

1. Introduction

Purpose of kit

Ribosomal RNA (rRNA) constitutes a significant portion, up to 90%, of the total RNA extracted from the majority of organisms. Thus, removing rRNA prior to sequencing enables a more sensitive and cost-effective detection of target RNAs.

The high-input rRNA depletion pool kit (riboPOOL kit – High-Input) developed by siTOOLs Biotech represents an efficient, affordable and flexible solution to deplete rRNA from 5 up to 30 µg of total RNA. It is indicated for all downstream applications that require large amounts of input RNA (e.g., Oxford Nanopore Direct RNA Sequencing). The only requirement for the total input RNA is an RNA Integrity Number (RIN) preferably above 7.

Ribodepletion with riboPOOLs can be completed within 70 minutes.

Product description

Composed of high complexity pools of optimally designed biotinylated DNA probes, riboPOOLs specifically hybridize with cytoplasmic, mitochondrial, and/ or plasmid rRNAs, enabling their removal with streptavidin-coated magnetic beads. Following the biotin-streptavidin magnetic bead-based removal of rRNAs, remaining RNA is cleared of salts and buffer concentrates via the Zymo RNA Clean & Concentrator Kit (not included).

riboPOOLs are available for a diverse array of organisms: https://www.sitoolsbiotech.com/products/ribopools/rrna-depletion/available-ribopools

Product performance

riboPOOLs were demonstrated by RNA-Seq to reliably deplete > 95 % rRNA with high reproducibility, and consistently recover mRNA and other RNAs of interest (Fig. 1).

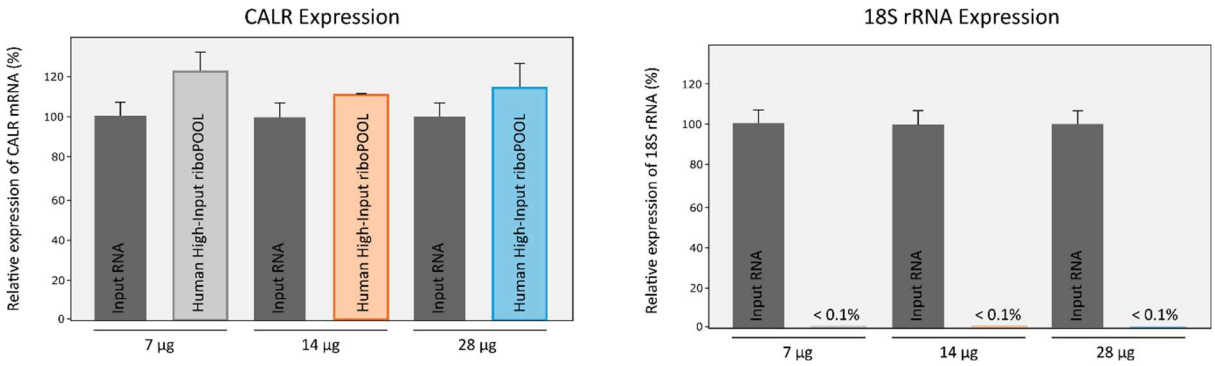

Fig 1. Expression of human calreticulin (CALR) mRNA **(Left)** and 18S rRNA **(Right)** before and after ribodepletion of 7, 14 and 28 µg of total input RNA with the Human High-Input riboPOOL kit. Detection of CALR mRNA, a widely expressed marker for mRNA, is not significantly affected by rRNA depletion.

2. Contents of High Input riboPOOL kit

Abbreviation and volume

Reagent	6 reaction kit	12 reaction kit	24 reaction kit
High-Input Hybridization Buffer H-HB	1 x 1 ml	1 x 1 ml	1 x 1 ml
High-Input Depletion Buffer H-DB	1 x 8 ml	2 x 8 ml	2 x 8 ml
H_2O – Nuclease-free water	1 x 1 ml	1 x 1 ml	1 x 1 ml
Streptavidin-coated magnetic beads	1x 3.3 ml	1x 6.6 ml	1x 13.2 ml
riboPOOL - RP*	1x lyophilized powder	1x lyophilized powder	1x lyophilized powder

Storage Instructions

The riboPOOL kit – High-Input is shipped at room temperature. Upon receipt, please store reagents at the following temperatures.

Reagent	Room temperature	4°C	-20°C	Notes
H-HB	X			stable for at least 1 year
H-DB	X			stable for at least 1 year
H_2O	X			stable for at least 1 year
SMB		X		stable for at least 1 year
RP*			X	stable for at least 1 year

*The riboPOOL (RP) - when lyophilized – is stable at room temperature for up to a year, but is best stored at -20°C upon receipt. Upon resuspension in nuclease-free water, riboPOOLs are stable for at least one year when stored at or below -20°C. Please store in aliquots to avoid freeze-thaw cycles.

3. Additional material required (not supplied in kit)

- Zymo RNA Cleanup and Concentrator Kit (Zymo Research #R1013, R1014, R1015, R1016)
- Thermo-Mixer or thermal cycler for PCR
- RNase inhibitor (optional)
- Sterile, low-retention pipette tips for minimal surface binding of RNA and beads
- Common laboratory equipment – benchtop centrifuge, vortex, pipettes
- 100% and 70% research-grade ethanol in H_2O
- Personal protection equipment – lab coat, gloves

4. Application tips

- Input RNA should be DNA-free with a RIN value above 7.
- Input RNA amount should be between 5 µg and 30 µg. For smaller amounts (1 -5 µg), we recommend using the standard input riboPOOL kit.
- Take necessary precautions to avoid RNase contamination i.e., keep work area clean, wear gloves, avoid leaving tubes open for prolonged periods of time.
- To agitate tubes containing streptavidin-coated magnetic beads, flick the tube gently till solution becomes homogenous. Alternatively, vortex the tube very briefly at medium speed.
- Follow recommended volumes and incubation temperatures. Modifying protocol parameters may result in decreased efficiency and RNA recovery.

5. High-Input riboPOOL Kit Protocol

Notes before starting:

- Make sure all reagents are equilibrated to room temperature before use.
- RNA samples should always be stored on ice until hybridization.
- Set heat block or thermal cycler to 68°C.

Preparation and Depletion

5.1 Resuspension of riboPOOL

a. Centrifuge riboPOOL (RP ●) at 11000x g for 30s before opening.

b. For respective kit size, add the following amount of nuclease-free water (H_2O ●) provided into RP tube (●).

Kit size	H_2O to add (µl)
6 reactions	45
12 reactions	90
24 reactions	180

c. Vortex well.

d. Spin down contents of tube before using.

5.1.1 (Special case) Combination riboPOOL

a. Pre-mixed
   - If the Combination riboPOOL was provided pre-mixed, follow instructions provided in Step 1 to resuspend the riboPOOL.
   - Proceed to Step 2.

b. Separate tubes
   - If each component of the Combination riboPOOL was provided in a separate tube, resuspend each riboPOOL as described in Step 1.
   - Prepare the Combination riboPOOL by adding the require amount of each riboPOOL to a new tube based on the desired final ratio.
   - Vortex well and proceed to Step 2.

5.2 Hybridization of riboPOOL to sample

a. Prepare the Hybridization mix in 0.2 ml PCR strip tubes.

b. To 113 µl of RNA sample (containing 5 µg – 30 µg of total RNA), add:

- 7.2 µl of resuspended RP (🟠) (from Step 1)
- 40 µl of High-Input Hybridization Buffer (H-HB 🟡)
- RNase inhibitor (optional)

  Follow manufacturer's instructions for volume required and ensure enzyme is active at 68°C. RNase inhibitor may also be introduced during bead preparation.

c. Vortex well and spin down droplets.

d. Place the samples in the PCR thermal cycler and incubate at 68°C for 10 minutes to denature the RNA.

e. Allow the samples to cool slowly from 68°C to 37°C for optimal hybridization.

   To do so, shut off the heating block and let temperature naturally fall to 37°C. If using temperature-controlled ramping, cool at a rate of 3°C/minute.

5.3 Preparation of the beads

a. Resuspend the streptavidin-coated magnetic beads (🔵) by carefully vortexing the tube at medium speed.

b. Transfer 540 µl of bead suspension per sample into a new 1.5 ml tube.

c. Place tube on magnetic rack and wait for 1 minute. Beads may stick to the side of the tube making the solution appear brown. Aspirated solution, however, should be clear.

d. Aspirate and discard the supernatant.

e. Add 640 µl of High Input - Depletion Buffer (H-DB ⚪) to each tube and carefully resuspend the beads.

f. Place the tube back on the magnetic rack and repeat steps 3c to 3e.

g. Place the beads at 50°C in a Thermo-Mixer (no shaking) until further use.

5.4 Ribosomal RNA depletion

a. Briefly centrifuge the PCR strip tubes containing the Hybridization mix and transfer the samples to 1.5 ml tubes.
b. Pipette half of the prepared beads (320 µl) into the tube containing the hybridized riboPOOL-RNA solution and resuspend carefully.
c. Incubate at 50°C in a Thermo-Mixer (no shaking) for 20 minutes.
d. Briefly spin down droplets.
e. Place the tubes on a magnet for 2 mins and carefully transfer the supernatant to a fresh tube.
f. Pipette the remaining beads (320 µl) into the tube containing the supernatant and resuspend carefully.
g. Repeat steps 4c to 4e.
h. Optional: place the tubes on the magnet again for 1 minute to remove any potential trace amounts of beads. Transfer the supernatant to a new 1.5 ml tube.

Safe point: samples can be stored at -20°C overnight or -80°C for up to a month.

5.5 RNA purification

To purify the sample, follow the protocol for the Zymo RNA Clean & Concentrator kit according to manufacturer instruction. If required, this kit allows the separation of RNAs into different fractions based on sequence length (approx. 17 – 200 nt, and > 200 nt). After addition of ethanol and binding buffer, the total sample volume will exceed the maximum loading capacity of a single Zymo column. Load the sample onto the same column in multiple aliquots: apply the first aliquot, centrifuge, discard the flow-through, and then repeat with the remaining volume. Only one Zymo column should be used per sample.

For the final step, elute the RNA in at least 13 µl of H_2O. If necessary, the elution volume may be reduced to 6 µl, however, overall RNA recovery might be lower.

Available riboPOOLs

Single species riboPOOLs

Aedes albopictus
Amphimedon queenslandica
Anaeramoeba flamelloides
Anopheles gambiae
Apis mellifera
Arabidopsis thaliana
Argiope bruennichi
Azolla filiculoides
Bacillus subtilis
Bemisia tabaci
Blood Parasites
Caenorhabditis elegans
Caulobacter crescentus
Chincilla lanigera
Chlamydomonas reinhardtii
Clostridium perfringens
Crassostrea gigas
Cryptococcus neoformans
Cupriavidus necator
Cyanidioschyzon merolae
Danio rerio
Deroceras leave
Drosophila melanogaster
Emiliania huxleyi
Escherichia coli
Eubacterium limosum
Gallus gallus
Haloferax volcanii
Human
Human Blood
Human-Mouse-Rat
Human rRNA-tRNA
Ixodes scapularis
Leptinotarsa decemlineata
Mouse-Rat
Nematostella vectensis
Olavius algarvensis
Oryza sativa
Pichia pastoris
Pinus sylvestris
Plautia stali
Pseudomonas aeruginosa
Saccharomyces cerevisiae
Salmonella enterica
Schistosoma mansoni
Schizosaccharomyces pombe
Schmidtea mediterranea
Spodoptera exigua
Staphylococcus aureus
Stenotrophomonas sp.
Strongyloides ratti
Thalassiosira pseudonana
Ustilago maydis
Varroa destructor
Wolbachia pipientis

Pan-riboPOOLs

Blood Parasite
Filamentous Fungi
Human-Holobiont
Pan-Actinobacteria
Pan-Aphid
Pan-Archaea
Pan-Bacteria
Pan-Bird
Pan-Chlamydia
Pan-Dictyostelia
Pan-Fungi
Pan-Mammal
Pan-Mussel
Pan-Plant
Pan-Prokaryote
Pan-Scleractinia
Pan-Symbiodiniaceae
Pan-Sponge
Seawater
Willaertia-Naegleria

Manual version and appropriate use

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The instructions within this manual should be strictly followed by qualified personnel for safe and proper use of the product(s) described herein. Failure to completely read and perform the protocol in an adequate test environment may result in damage to the product(s), injury to persons, including to users or others, and damage to other property. siTOOLs Biotech does not assume any liability arising out of the improper use of the product(s) in any form or environment.

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The riboPOOL kit is developed, designed, produced, and sold FOR RESEARCH PURPOSES only. No claim or representations is intended for clinical use (included, but not limited to diagnostic, prognostic, therapeutic purposes). It is rather the responsibility of the user to inspect and assure the use of the riboPOOL kit for a well-defined and specific application.

For other general terms of business and safety documentation, please refer to the siTOOLs Biotech website (www.sitoolsbiotech.com) under Resources

This manual, referred to hereby as High_Input_riboPOOLKitManual_v1, was first created on 07th January 2025. It may be subject to future revisions. Please refer to our website for latest updates

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