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We drive RNA research with tailored molecular tools

riboPOOL™ Kit Manual

Ribosomal RNA depletion
and RNA purification kit

Catalog No. dp-K006, dp-K012, dp-K024, dp-K096

Content

Introduction

Purpose of kit

Ribosomal RNAs (rRNAs) constitute a significant portion, up to 90%, of the RNA extracted from the majority of organisms. This applies to RNA samples used in both ribosome profiling (Ribo-Seq) and total RNA-Seq analyses. To enable sensitive and cost-effective detection of target RNAs, removal rRNAs prior to sequencing is thus necessary.

Ribosomal RNA depletion pools for ribosome profiling (Ribo-Seq riboPOOLs) developed by siTOOLs Biotech represent an efficient, affordable, and flexible solution offering scientists the freedom to deplete rRNAs from any species, thereby increasing abundance of RNAs of interest. Ribo-Seq riboPOOLs can be utilized for rRNA depletion in both Ribo-Seq and total RNA-Seq samples, allowing to use the same reagents when processing samples for both workflows.

Since all riboPOOLs are specifically optimized for their respective application, we do recommend using Ribo-Seq riboPOOLs for ribosome profiling and 'Standard RNA-Seq' riboPOOLs for common RNA-Seq workflows of high-quality RNA (RIN>7).

Ribodepletion with Ribo-Seq riboPOOLs can be completed within 70 minutes, without the need for enzymes, and is compatible with high-throughput automation.

Product description

Composed of high complexity pools of optimally designed biotinylated DNA probes, Ribo-Seq riboPOOLs specifically hybridize with cytoplasmic 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 by either ethanol precipitation (recommended for Ribo-Seq) or SPRI bead-based purification (recommended for total RNA-Seq). All Ribo-Seq riboPOOL kits include reagents for both purification approaches.

Ribo-Seq riboPOOLs are available for a diverse array of organisms (Table 1).

Product performance

Ribo-Seq riboPOOLs can efficiently deplete rRNAs from ribosome profiling samples, leading to more than 300% increases in the abundance of RPFs (Figure 1-3), while providing an unaltered overview of the transcriptome (Figure 2).

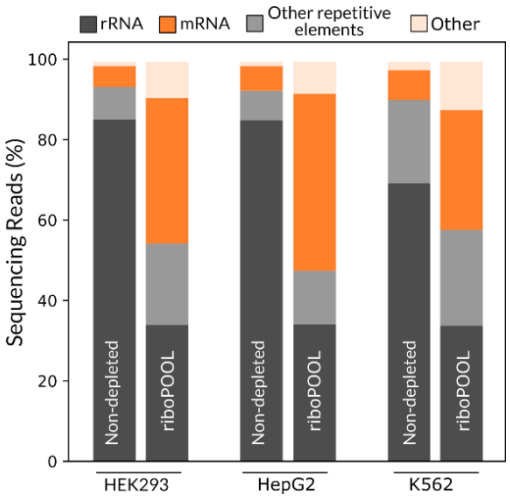


Fig. 1 Percentages of sequencing reads mapping to different RNA categories in non-depleted and depleted samples from three different cell lines.

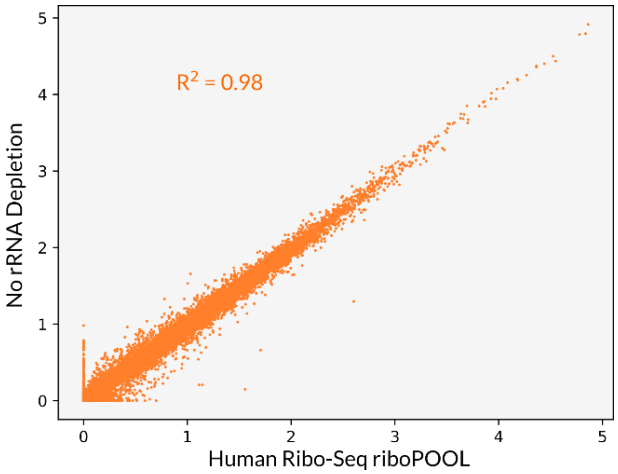


Fig. 2 Abundance (log10 RPKM) of coding transcripts in non-depleted and rRNA depleted samples of HEK293 cells.

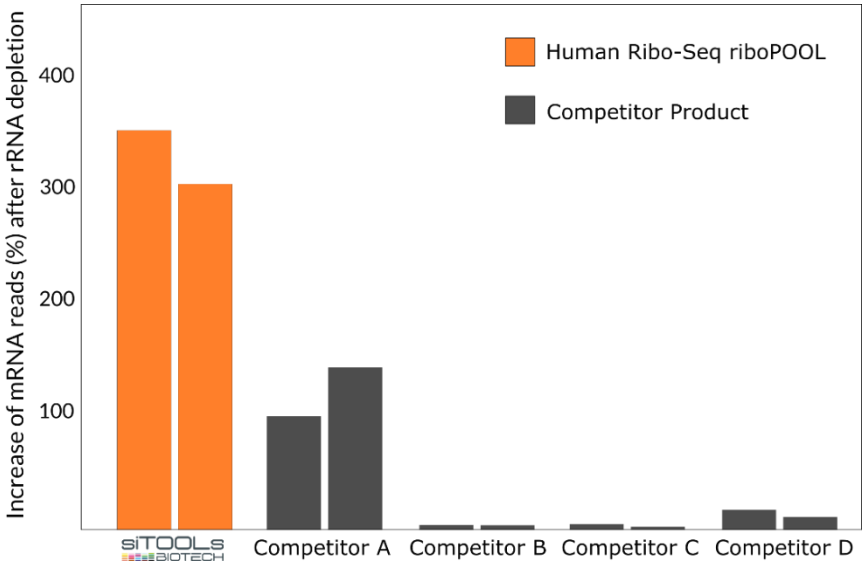


Fig. 3 Percentage increase of reads mapping to mRNA after rRNA-depletion with Human Ribo-Seq riboPOOL (orange) and competitor products (dark gray).

Contents of riboPOOL kit

Abbreviation and Volume

Code	Component	6 reaction kit (Trial)	12 reaction kit	24 reaction kit	96 reaction kit
RP	riboPOOL	1 x lyophilized powder	1 x lyophilized powder	1 x lyophilized powder	1 x lyophilized powder
H_2O	Nuclease-free water	1 x 1 ml	1 x 1 ml	1 x 1 ml	1 x 1 ml
HB	Hybridization buffer	1 x 1 ml	1 x 1 ml	1 x 1 ml	1 x 1 ml
DB	Depletion buffer	1 x 8 ml	1 x 8 ml	1 x 8 ml	2 x 8 ml
SMB	Streptavidin-coated magnetic beads	1 x 0.6 ml	1 x 1.2 ml	2 x 1.2 ml	8 x 1.2 ml
LA	Linear acrylamide	1 x 30 µl	1 x 30 µl	1 x 30 µl	1 x 110 µl
SA	Sodium acetate, 3M	1 x 300 µl	1 x 300 µl	1 x 300 µl	1 x 1.1 ml
CB*	Clean-up beads	1 x 1050 µl	1 x 2 ml	1x 4ml	1x 16 ml

* Use of clean-up beads is not recommended for Ribo-Seq experiments as it can lead to loss of low recovery of ribosome protected fragments (RPFs).

Storage instructions

The riboPOOL kit is shipped at room temperature.

Upon receipt, please store reagents at the following temperatures:

Reagents	Room temperature	4°C	20°C	Notes
HB	X			stable for at least 1 year
DB	X			stable for at least 1 year
SA	X			stable for at least 1 year
SMB		X		stable for at least 1 year
CB		X		stable for at least 1 year
LA			X	stable for at least 1 year
RP*			X	stable for at least 1 year

*The riboPOOL (RP) when lyophilized, is stable at RT for up to a year, but is best stored at -20°C upon receipt. Upon resuspension in nuclease-free water (H_2O), riboPOOLs are stable for at least one year when stored at or below -20°C. Please store in aliquots to avoid freeze-thaw cycles.

Additional material required

(not supplied in kit)

- Magnetic rack or plate
- Temperature-controlled mixer or thermal cycler
- RNase inhibitor (optional)
- Sterile, low-retention pipette tips for minimal surface binding of RNA and beads
- 96-well PCR plate and sealing foil or caps (for 96 reaction kit)
- Common laboratory equipment – benchtop centrifuge, vortex, pipettes
- 100% research-grade ethanol
- 70% research-grade ethanol
- Personal protection equipment – lab coat, gloves

Application tips

Ribo-Seq

- Perform ribodepletion after RNA purification following on-gel RNA size selection. Using linker-ligated RNA as input or performing the ribodepletion prior to on-gel RNA size-selection can lead to decreases in rRNA-depletion efficiencies.
- Avoid using SPRI beads for post-depletion RNA purification as it can lead to loss of RPFs.

General

- RNA input should be DNA-free.
- RNA input amount may range from 100 ng to 5 µg. For larger amounts, split into aliquots ≤ 5 µg and follow protocol accordingly.
- Take necessary precautions to avoid RNase contamination i.e. keep work area clean, wear gloves, leave tubes open for prolonged times.
- Equilibrate all reagents to room temperature before use.
- To agitate tubes containing streptavidin magnetic beads, flick the tube gently till solution becomes homogenous. Alternatively, vortex the tube at medium speed.
- Follow recommended volumes of reagents for RNA input 100 ng – 5 µg as modifying volumes may result in decreased efficiency. For input amount < 100 ng, reduce probe amount to 30 pmol/reaction.
- Be sure to resuspend clean-up beads completely before using them. The beads should appear homogenous and consistent in color.
- Avoid touching the pellet containing your RNA when removing the supernatant during the last wash step.
- Make sure all the supernatant is removed after the last wash step.

riboPOOL Kit Protocol

Notes before starting:

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

Preparation and Depletion

1. Resuspension of riboPOOL RP, H_2O (Kit components required)

a. Centrifuge **riboPOOL (RP)** at 11 000 x *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 reaction (1 nmol)	10
12 reaction (1.5 nmol)	15
24 reaction (3 nmol)	30
96 reaction (11 nmol)	110

c. Vortex well.

d. Spin down contents of tube before using.

1.1 (Special case) Combination riboPOOL

a) Pre-mixed

- If the Combination riboPOOL was provided as 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 required amount of each riboPOOL to a new tube based on the desired final ratio.

- Vortex well and proceed to section 2.

2. Hybridization of riboPOOL to RNA RP, HB, RNA sample

a. To 14 µl of RNA sample (containing 100 ng - 5 µg of total RNA), add:
    a. *If sample volume is > 14 µl, adjust HB volume accordingly to 0.25X total volume. Total volume however should not exceed 40 µl.*
    - 1 µl of **resuspended RP** (from step 1; i.e. 100 pmol)
    - 5 µl of **Hybridization Buffer (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.

b. Vortex well and spin down droplets.

c. Incubate at **68°C for 10 min** to denature RNA.

d. Allow to **cool slowly** from **68°C to 37°C** for optimal hybridization.
    a. *To do this, shut off the heating block and let temperature fall naturally to 37°C. If using temperature-controlled ramping, cool at 3°C/min.*

3. Preparation of beads SMB, DB

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

b. Transfer 90 µl of bead suspension per sample into a fresh tube.
   For batch washing of beads for multiple samples, aliquot bead suspension for up to 6 (i.e. 540 µl) or 12 samples (i.e. 1080 µl) in a single tube.

c. Place tube on magnetic rack and wait for 1 min.
   Beads may stick to sides of tube making solution appear brown. Aspirated solution, however,

should be clear.

d. Aspirate and discard all supernatant.

e. Add 80 µl per sample (i.e. 480 µl for 6 samples, 960 µl for 12 samples) of **Depletion Buffer (DB)** and agitate the tube well to resuspend beads.

f. Repeat steps c to e.

4. Ribosomal RNA depletion

a. Briefly centrifuge the tube containing ~20 µl hybridized riboPOOL and total RNA (from step 2) to spin down droplets.

b. Pipette 80 µl of the prepared beads (from step 3) into the tube containing hybridized riboPOOL-RNA solution. Agitate the tube to resuspend well.

c. Incubate the tube at **37°C for 15 min**, followed by a **50°C incubation for 5 min**.

d. Briefly spin down droplets.

e. Place on magnet for 2 min then carefully transfer the supernatant to a new tube.

f. Place tube on magnet for 1 min to get rid of trace amounts of beads.

g. Carefully transfer the supernatant to a new tube.
Steps f and g are recommended to remove any potential trace amount of beads but can be left out if desired.

At this point, RNA can be stored at -20°C overnight or -80°C for up to a month.

5. Proceed to RNA purification with clean-up beads (option A) or ethanol precipitation (option B)

RNA Purification

RNA samples subject to rRNA depletion **must** be purified before sequencing library preparation to remove salts and buffer concentrates.

The choice of clean-up method may employ a size-selection of RNA fragments that can affect sequencing results. The riboPOOL kit includes reagents for ethanol precipitation (recommended for Ribo-Seq samples) which will recover RPFs.

RNA clean-up beads or SPRI bead-based RNA purification may recover RNA fragments > 200 nt (which excludes tRNAs and small RNAs) dependent on specific buffer conditions used.

Option A: Ethanol Precipitation (Ribo-Seq and total RNA-Seq)

The workflow with the riboPOOL™ cleanUP module can be completed in ~30 min, is enzyme-free, and compatible with high-throughput automation.

1. Add 90 µl of your RNA sample (e.g. rRNA depleted RNA) in an appropriate tube.
2. Add 162 µl **Clean-up Beads (CB)** to each sample. Be sure to resuspend the **beads** completely before adding them by flicking the tube or by pipetting.
   Note: The volume of Clean-up Beads for a given reaction can be calculated from the following equation:
   Volume of Clean-up Beads per reaction = 1.8 x reaction volume.
3. Mix solution with 6-8 pipetting strokes and let the tube incubate at room temperature for 5 minutes. This step binds RNA products to the magnetic beads. Vortex is not recommended.
4. Separate the **Clean-up Beads** by placing the tube on a magnet for 5 minutes. The separation time is dependent on size. Larger reactions will take longer to separate. Wait for the solution to clear before proceeding to the next step.
5. With the tube still on the magnet, slowly remove and discard the supernatant without disturbing the pellet.
6. With the tube still on the magnet, add 500 - 1000 µl of 70% ethanol (70% ethanol and 30% H_2O) to each sample and incubate for 30 seconds at room temperature. Carefully remove and discard the ethanol without disturbing the pellet.

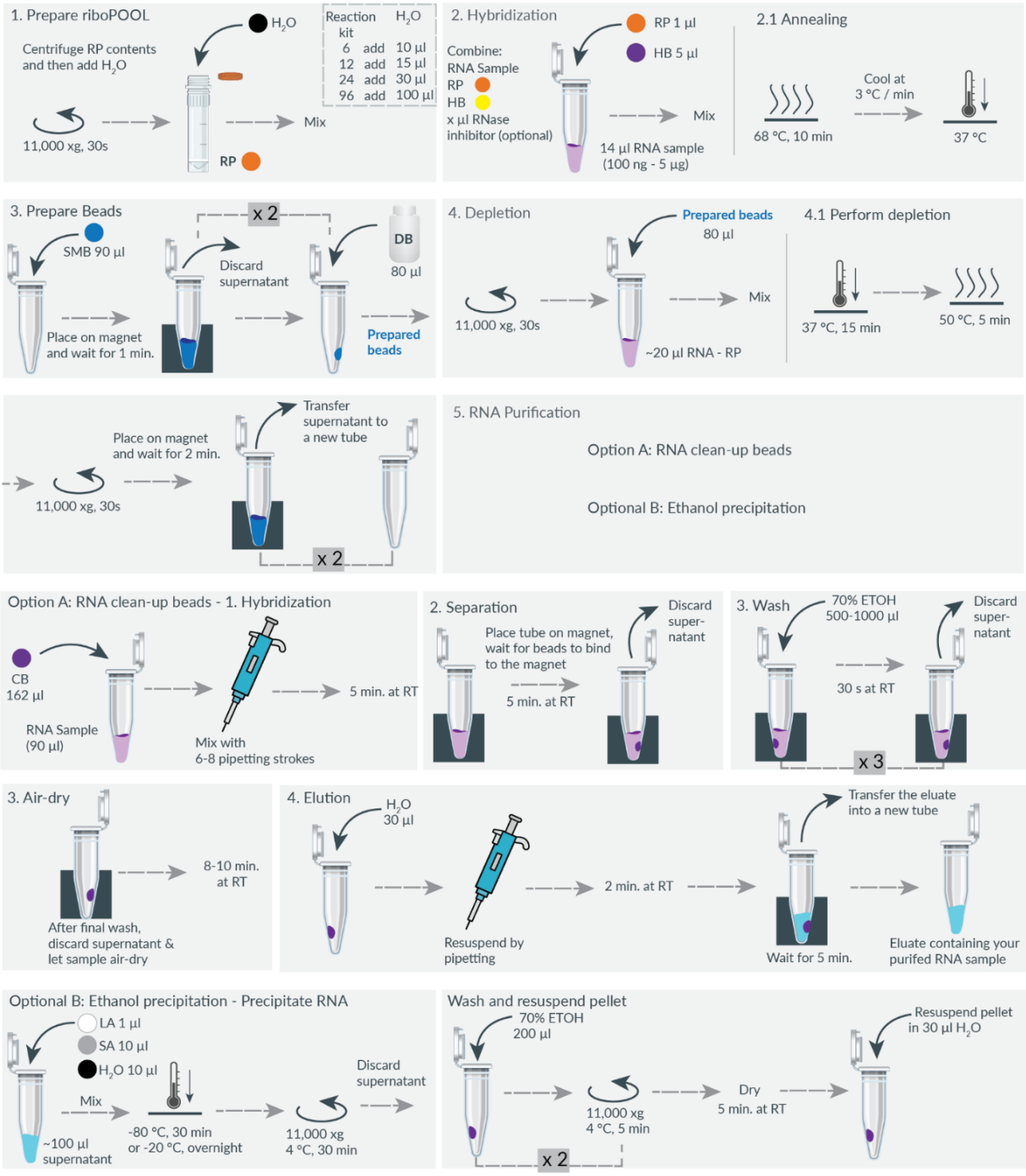
Repeat for a total of three washes. Note: Be sure to remove all ethanol from the tube as it may contain residual contaminants. The wash solution should completely cover the pellet in the tube.

7. Let the reaction tube air-dry for 10 minutes on the magnet. The tube should air-dry until the last visible traces of ethanol evaporate. Be careful and do not over dry the pellet as it may lead to lower recovery.
8. Remove the tube from the magnet. Elute the purified RNA from the beads by adding at least 30 µL of nuclease-free water. Resuspend the beads by pipetting up and down several times. Elution volume can be modified if higher/lower RNA concentration is needed, but it's not recommended to eluate in less than 10 µl.
9. Incubate for 2 min. at room temperature.
10. Place the tube back on the magnet and wait for 5 minutes or until the **Clean-up Beads** clear from solution.
11. Transfer the eluate containing your purified RNA sample to a clean tube.

Option B: Clean-up beads purification (total RNA-Seq)

1. Add 10 µl of **3M sodium acetate (SA)**, 1 µl of **Linear acrylamide (LA)** and 333 µl of 100% ethanol.
2. Vortex well.
3. Incubate tube at -80°C for 30 min OR -20°C overnight.
4. Centrifuge at 11 000 x *g* (or max speed) for 30 min at 4°C
5. Carefully remove and discard all supernatant, making sure not to disrupt pellet.
6. Add 200 µl of 70% ethanol to wash pellet.
7. Centrifuge at 11 000 x g for 5 min at 4°C.
8. Carefully remove and discard all supernatant, making sure not to disrupt pellet.
9. Repeat ethanol wash (steps 6 to 8).
10. Dry pellet at room temperature for 5 min.
11. Resuspend the pellet in 30 µl of nuclease-free water (H_2O) or appropriate elution buffer for library preparation. If required elution volume can be decreased down to 10 µl.

Snapshot Protocol



List of Available Ribo-Seq riboPOOLs

Single Species Ribo-Seq riboPOOLs
Acanthoamoeba castellani
Arabidopsis thaliana
Caenorhabditis elegans
Danio rerio (Zebrafish)
Drosophila melanogaster
Ectocarpus siliculosus
Escherichia coli
Homo sapiens
Leishmania mexicana
Pristionchus pacificus
Saccharomyces cerevisiae (Yeast)
Toxoplasma gondii
Trypanosoma brucei

Human-Mouse-Rat Ribo-Seq riboPOOLs
Human – Mouse - Rat
Mouse - Rat

Add-ons (can be added to any human or mouse Ribo-Seq riboPOOL)
Human tRNAs
Mouse tRNAs
Signal Recognition Particle (7SL)

Manual version and appropriate use

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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 > Other Downloads.

This manual, referred to hereby as RiboSeq_riboPOOLKitManual_v2, was first created on 30th September 2019 and revised on 13th November 2019, on 11th February 9th July 2020, 16th December 2021, December 2022, March 2023 and March 2024. It may be subject to future revisions. Please refer to our website for latest updates.

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