rRNA Depletion Kits (Pan-Bird)

RK30240



Instruction Manual

Version: N19D28v1.0

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1. Overview

rRNA Depletion Kits (Pan-Bird) (ABclonal, Cat. RK30240) is an efficient rRNA removal kit specifically designed for bird. This kit is suitable for bird total RNA samples with a template amount ranging from 10ng to 1000ng. It effectively removes rRNA (including cytoplasmic rRNA and mitochondrial rRNA) through streptavidin magnetic bead adsorption, while retaining other mRNAs and non-coding RNAs for subsequent research applications such as whole transcriptome sequencing and long non-coding RNA sequencing.

The kit's workflow consists of four main steps: hybridization of Ribo-Pools probes with target RNA, pre-treatment with streptavidin magnetic beads, rRNA removal, and RNA purification. The entire process can be completed within 60 minutes without the need for enzymatic digestion and is compatible with high-throughput automation.

Each reagent in the kit undergoes strict quality control, and every batch of the library preparation kit is validated through library construction and sequencing, ensuring the stability of the kit's performance.

2. Kit Compents

	Tube Name	4 RXN	96 RXN
RK30240 (2-1)	Probe Mix	13.5 μL	317 μL
RK30240 (2-2)	Hybridization buffer	25 μL	600 μL
	Depletion buffer	800 μL	18.5 mL
	Streptavidin-coated magnetic beads	176 μL	4.23 mL
	Purification beads	795 μL	19 mL

3. Storage

Module Name	Storage
RK30240 (2-1)	-20℃
RK30240 (2-2)	4℃

4. Additional Materials

Freshly prepared 80% ethanol.

Magnetic rack.

PCR system.

5. Precautions

5.1. Input RNA should be free of DNA contamination.

5.2. During operation, avoid opening the RNA tube lids (during incubation) or leaving them at room temperature for extended periods.

5.3. Take necessary precautions to avoid RNase contamination, such as keeping the work area clean, wearing gloves, and not speaking or working over open tubes.

5.4. After rRNA removal, due to the high abundance of rRNA, an expected loss of ≥ 95% of the initial RNA amount is anticipated.

5.5. Bring all reagents to room temperature before use.

5.6. Magnetic beads must be thoroughly mixed before use.

6. Protocols

6.1. Probe/rRNA Hybridization

Preparation: Thaw the Probe Mix on ice and mix well. Restore the Streptavidin-coated magnetic beads and Hybridization buffer to room temperature.

6.1.1. Take 10-1000 ng of total RNA and dilute it with Nuclease-free water to a final volume of 12 μL. Keep it on ice for later use.

6.1.2. Add each component sequentially according to the table below:

Reagent	Volume
Hybridization buffer	5 μL
Probe Mix	3 μL
Total RNA	12 μL
Total Volume	20 μL

Note: If sample volume exceeds 12 μL, adjust the Hybridization buffer volume accordingly to maintain a 0.25× ratio of the total reaction volume. The final volume must not exceed 40 μL.

6.1.3. Gently mix by pipetting up and down 15-20 times, and centrifuge briefly. Place the tube in a thermocycler, and run the following program (Lid preheated to 105℃).

Temperature	Time
68℃	10 min
68-37℃	0.1℃/sec
37℃	Proceed to the next step immediately*

Note: After adopting gradient annealing, it is necessary to quickly proceed to step 6.3. Holding at 4℃ in the PCR program is strictly prohibited, as it will affect the removal efficiency.

6.2. Pre-treatment of Streptavidin-coated magnetic beads

6.2.1. Vortex the Streptavidin-coated magnetic beads (equilibrated to room temperature) to ensure thorough mixing.

6.2.2. Transfer 40 μL of Streptavidin-coated magnetic beads per sample into a new RNase-free PCR tube.

Note: For multiple samples, beads can be washed simultaneously. For example, pool beads from 6 (240 μL) or 12 samples (480 μL) in a single tube for washing.

6.2.3. Place the PCR tube on the magnetic rack stand for ~2 min until the solution clears. Carefully remove the supernatant without disturbing the beads.

6.2.4. Remove the PCR tube from the magnetic rack, add 80 μL of Depletion buffer per sample, and vortex to mix.

6.2.5. Repeat steps 6.2.3 to 6.2.4.

6.3. rRNA depletion

6.3.1. Centrifuge the product from step 6.1.3 briefly to collect the solution at the bottom of the tube, add 80 μL of pre-treated Streptavidin-coated magnetic beads (Step 6.2.5) and mix by pipetting.

6.3.2. Incubate at room temperature for 15 min, with vortex mixing every 5 min during the incubation.

6.3.3. Then place it in the PCR machine (with the heated lid set to 105℃) to run the following program:

Temperature	Time
50℃	5 min
Proceed to the next step immediately*	

Note: After completing the removal reaction program, it is essential to proceed to the next operational step as quickly as possible. Holding at 4℃ in the PCR program is strictly prohibited, as it will affect the removal efficiency.

6.3.4. After the reaction is complete, immediately place the sample tube on the magnetic rack and let it sit for about 2 minutes.

6.3.5. Once the solution becomes clear, carefully transfer the supernatant (~100 μL)to a new RNase-free PCR tube, taking care not to aspirate the beads.

Safe stop point: The product can be stored at -20℃ overnight or at -80℃ for one month.

6.4. RNA Purification

6.4.1. Remove the purification beads in advance and equilibrate at room temperature for 30 min, vortex to mix thoroughly before use.

6.4.2. Pipette 180 μL of purification beads into the product from step 6.3.4, mix by pipetting, and let it stand at room temperature for 5 min.

6.4.3. Transfer the centrifuge tube to a magnetic rack for approximately 5 min until the solution becomes clear, then discard the supernatant (be careful not to aspirate the magnetic beads).

6.4.4. Keep the centrifuge tube on the magnetic rack, add 200 μL of freshly prepared 80% ethanol, let it sit for 30 s, and discard all the supernatant.

6.4.5. Repeat step 6.4.4 (repeat washing once).

6.4.6. Keep the tube on the magnetic stand, remove residual ethanol at the bottom using a 10 μL pipette, and air-dry the beads at room temperature for 2-3 min with the lid open until complete ethanol evaporation (*the color of the beads changes from shiny brown to matte brown*).

6.4.7. Depending on the experimental purpose, choose different steps:

A. If used for RNA library construction: Refer to the RNA library construction manual (ABclonal, Cat. RK20306) and add 1X Frag/Elute Buffer, then immediately proceed with the subsequent library construction steps.

B. If the eluted product is used for other experiments: Remove the sample tube from the magnetic rack, add 10-15 μL of RNase-free water, mix by pipetting, and incubate at room temperature for 5 min. Then transfer the centrifuge tube to the magnetic rack and let it sit for 1 min. Once the solution becomes clear, carefully transfer the supernatant to a new RNase-free PCR tube for subsequent experiments or store at -80℃.

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