GeneRead™ rRNA Depletion Nano Kit, Part 1

The GeneRead rRNA Depletion Nano Kit (cat. no.180224) is shipped on ice. Upon receipt, store HMR rRNA Depletion Probes, Antibody Solution, Hybridization Buffer, and BioMag Protein G Beads at 2–8°C. RNase Inhibitor should be stored at –20°C. All other kit components can be stored at room temperature (15–25°C). When stored under these conditions and handled correctly, the product can be kept for up to 12 months without showing any reduction in performance.

Further information

- *GeneRead rRNA Depletion Handbook*: www.qiagen.com/handbooks
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: toll-free 00800-22-44-6000, or www.qiagen.com/contact

Notes before starting

- This protocol is for rRNA depletion using the GeneRead rRNA Depletion Nano Kit.
- Remove all kit components from the refrigerator or freezer.
- Preheat the heater and heater/shaker to 70°C and 50°C, respectively.

Table 1. Composition of the GeneRead rRNA depletion mix

Component	Volume per reaction (<500 ng RNA)	Volume per reaction (0.5–1 µg RNA)
Hybridization Buffer	5 µl	5 µl
Purified total RNA	100 ng to <500 ng in 1–36 µl	0.5–1 µg in 1–34 µl
HMR rRNA Depletion Probes	5 µl	5 µl
Antibody Solution	3 µl	5 µl
RNAse Inhibitor	1 µl	1 µl
RNase-Free Water	Variable	Variable
Total volume per reaction	**50 µl**	**50 µl**

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1. Mix BioMag Protein G Beads by vortexing for 15 s. For each sample, aliquot either 30 µl (for a starting amount of <500 ng total RNA) or 45 µl (for a starting amount of 0.5–1 µg total RNA) of BioMag Protein G Beads into a separate 1.5 ml reaction tube (supplied). Place the reaction tube on a magnetic rack or stand for a minimum of 2 min. Remove and discard the supernatant. Retain the bead pellet in the reaction tube.

2. For each sample, prepare a depletion mix according to Table 1 (see previous page) in a separate 1.5 ml reaction tube (supplied).

3. Mix the depletion mix thoroughly and incubate at 70°C for 5 min. After incubation, centrifuge the reaction mix briefly in a microcentrifuge to ensure that all the liquid is at the base of the tube.

4. Transfer the depletion mix to the bead pellet from step 1. Vortex briefly to resuspend beads, and incubate at 50°C with continuous shaking at 900 rpm for 30 min. **Optional**: If a heated shaker is not available, the depletion mix can be incubated at 50°C in a standard heat block. In this case, intermittently vortex the depletion mix and return to heat, no less than once every 5 min.

5. Transfer the depletion mix to the small spin column in a 1.5 ml collection tube (supplied). Close the lid gently and centrifuge at ≥8000 x *g* (≥10,000 rpm) for 30 s. Remove the small spin column from the collection tube and discard. The flow-through contains the depleted RNA sample. **Optional**: If magnetic separation is desired, remove the small spin column from the included collection tube. Place the depletion mix on a magnetic rack or stand for a minimum of 2 min. Transfer the supernatant, being careful not to disturb the bead pellet, to the collection tube. The supernatant contains the depleted RNA sample.

6. Clean up the depleted RNA sample using the RNeasy® MinElute® Cleanup Kit (supplied) as described in part 2 of the protocol.