NEXTFLEX® RiboNaut™ rRNA Depletion Kit

(Human / Mouse / Rat)

For 5 ng – 1 µg total RNA inputs

KIT CONTAINS: 8, 48, or 96 RXNS

(Compatible with Illumina® and Element® platforms)

USER MANUAL FOR :
#NOVA-512961
#NOVA-512962
#NOVA-512963

NEXTFLEX® RiboNaut™ rRNA Depletion Kit (Human / Mouse / Rat)

GENERAL INFORMATION

Product Overview

The NEXTFLEX® RiboNaut™ rRNA Depletion Kit (Human / Mouse / Rat) is designed to be compatible with human, mouse, and rat total RNA. The method used in the NEXTFLEX® RiboNaut™ rRNA Depletion Kit is based on subtractive hybridization, using a mixture of biotinylated oligonucleotides complementary to rRNAs. After hybridization of the bait oligos to total RNA, the rRNA/bait complex and excess bait oligos are removed by capture onto streptavidin-coated magnetic beads. The remaining RNA, which has been depleted of both cytoplasmic and mitochondrial rRNAs, is then purified and concentrated using magnetic beads.

Input amounts of total RNA ranging from 5 ng to 1 µg can be processed. The kit has been designed for use with human RNA; however, due to the nature of the probes, depletion of rRNA from mouse and potentially other mammalian species is also possible using this kit. RNA extracted from formalin fixed paraffin embedded (FFPE) samples may also be used. RNA-seq libraries made with rRNA-depleted intact RNA processed using this kit typically comprise less than 1% of the reads obtained in RNA-seq experiments. rRNA reads from highly degraded or FFPE total RNA samples may be higher. We recommended pairing the NEXTFLEX® RiboNaut™ rRNA Depletion Kit (H/M/R) with the NEXTFLEX® Rapid Directional RNA-Seq Kit 2.0.

Kit Contents, Storage & Shelf Life

This kit contains enough material to deplete rRNA from 8, 48, or 96 total RNA samples of 5 ng - 1 µg total RNA for use with RNA-Seq applications. The shelf life of all reagents is 12 months when stored properly.

Kit Contents	Cap Color	Amount (8/48/96 rxn)	Storage Temp.
NEXTFLEX® Bait Hybridization Buffer	PINK CAP	32/192/384 µL	Room Temp
NEXTFLEX® Cleanup Buffer	PINK CAP	760 µL/6 mL*/ 12 mL*	4°C
NEXTFLEX® RiboNaut™ Bait Mix	PINK CAP	40/240/480 µL	-20°C
NEXTFLEX® Cleanup Beads XP	BROWN CAP	160 µL/1.5 mL/ (2) 1.5 mL	4°C
NEXTFLEX® RiboNaut™ Beads	CLEAR CAP	(2) 1/12*/24* mL	4°C
Nuclease-free water	WHITE CAP	1.5/ 1.5/ (2) 1.5 mL	Room Temp
NEXTFLEX® RiboNaut™ Beads Wash Buffer	CLEAR CAP BOTTLE	5/26/52 mL	Room Temp

** in WHITE CAP BOTTLES*

Required Materials Not Provided

- 5 ng - 1 µg total RNA
- 96-well PCR plate magnetic stand
- 1.5 mL – 2 mL magnetic rack (optional)
- 15 mL tube magnetic rack (optional)
- 50 mL tube magnetic rack (optional)
- Thermal cycler
- 80% Ethanol
- 10, 20, 200 µL pipettes
- RNase-free pipette tips
- 96-well PCR plate
- 96-well PCR plate seals
- Microcentrifuge
- Vortex

Warnings & Precautions

- We strongly recommend that you read the following warnings and precautions. Periodically, optimizations and revisions are made to the components and manual. Therefore, it is important to follow the protocol included with the kit. If you need further assistance, you may contact your local distributor, or contact us at https://www.revvity.com/contact-us/technical-support and choose the "Next Gen Sequencing" category.
- Do not use the kit past the expiration date.
- Try to maintain a laboratory temperature of 20°–25°C (68°–77°F).
- RNA sample quality may vary between preparations. High-quality RNA should have a 28S band that is twice as intense as the 18S band of ribosomal RNA.
- Vortex beads until they are a uniform suspension prior to each use.
- Keep beads in liquid suspension during storage and handling. Never freeze beads.
- Ensure beads pellet on magnet before removing clear supernatant. Completely remove all washing buffer before eluting rRNA depleted RNA.

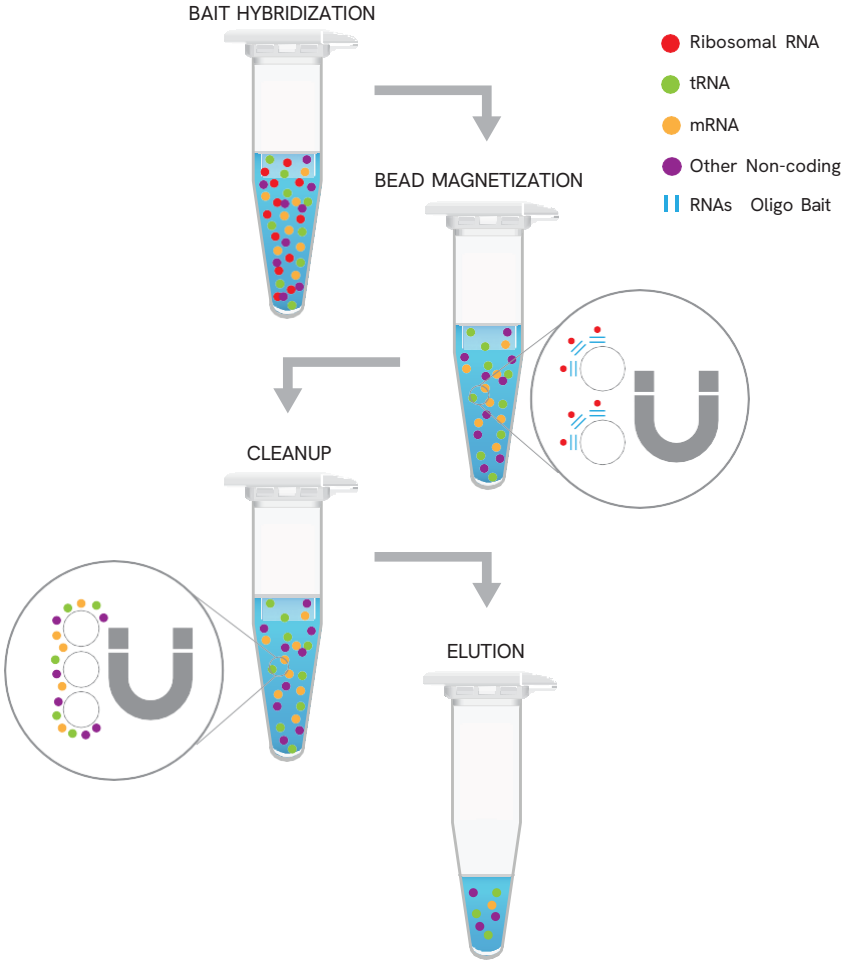
Revision History

Version	Date	Description
V19.12	December 2019	Product Launch
V20.03	March 2020	Clarifying language added. No protocol change.
V20.10	October 2020	Protocol change. Increased incubation times for Step B #3, #4, and Step C #3 for improved rRNA depletion efficiency.
V23.10	October 2023	Revvity rebranding

Starting Materials

The NEXTFLEX® RiboNaut™ rRNA Depletion Kit have been optimized and validated using 5 ng – 1 µg of human, mouse, and rat total RNA for RNA-seq applications. Best results are obtained by starting with high quality total RNA. RNA should be free of DNA, divalent cations, salts such as guanidinium salts, organics such as phenol, reducing agents such as DTT, and chelators such as EDTA. Revvity recommends quantification of total RNA inputs by Qubit® RNA HS assay (Thermo Fisher® Scientific). We also recommend examining total RNA integrity using a Revvity® LabChip® GXII Touch HT or equivalent. High quality or intact total RNA should have an RNA Quality Score (RQS) or RNA Integrity Number (RIN) ≥ 7. Partially degraded total RNA should have a RQS/RIN of 6.99 – 3. Highly degraded or FFPE total RNA should have an RQS/RIN of < 3. Additionally, highly degraded samples should have a DV200 greater than 50% for optimal RNA-seq results.

SAMPLE PREP WORKFLOW



SAMPLE PREP PROTOCOL

Step A: NEXTFLEX® RiboNaut™ Beads Preparation Procedure

MATERIALS

CLEAR CAP - NEXTFLEX® RiboNaut™ Beads

WHITE CAP BOTTLE - NEXTFLEX® RiboNaut™ Bead Wash Buffer

User Supplied

- 96-well PCR plate magnetic stand
- 1.5 mL – 2 mL magnetic rack (optional)
- 15 mL tube magnetic rack (optional)
- 50 mL tube magnetic rack (optional)

! NOTE: NEXTFLEX® RiboNaut™ Beads have a high sedimentation rate. Be sure to mix beads until homogenous before each pipetting step.

Beads preparation procedure in 96-well PCR plate:

1. Thoroughly resuspend NEXTFLEX® RiboNaut™ Beads until homogenous.
2. Add 200 µL of NEXTFLEX® RiboNaut™ Beads to each well.
3. Place the plate on the magnetic stand for 30 seconds.
4. Remove and discard supernatant taking care not to disturb the bead pellet.
5. Resuspend pellet in 200 µL of NEXTFLEX® RiboNaut™ Bead Wash Buffer.
6. Place the plate on the magnetic stand for 30 seconds.
7. Remove and discard supernatant taking care not to disturb the beads.
8. Add 45 µL of NEXTFLEX® RiboNaut™ Bead Wash Buffer to the pellet.

! NOTE: Bead pellet has a volume or displacement of 20 µL. Washed bead volume after resuspension with bead wash buffer should be 65 µL.

9. Proceed to Step B: Hybridize Baits to rRNA.

Beads preparation procedure in 1.5 mL – 2 mL, 15 mL, or 50 mL tube magnetic rack:

1. Thoroughly resuspend NEXTFLEX® RiboNaut™ Beads until homogenous.
2. For one sample, add 200 µL of NEXTFLEX® RiboNaut™ Beads to a 1.5 mL, 2 mL, 15 mL, or 50 mL tube. For 5 samples, add 1000 µL of NEXTFLEX® RiboNaut™ Beads to a 1.5 mL, 2 mL, 15 mL, or 50 mL tube. Scale up or down accordingly.
3. Place the tube containing the NEXTFLEX® RiboNaut™ Beads on the magnetic stand for 30 seconds.
4. Remove and discard supernatant taking care not to disturb the bead pellet.
5. For one sample, resuspend the pellet in 200 µL of NEXTFLEX® RiboNaut™ Bead Wash Buffer. For 5 samples, resuspend the pellet in 1000 µL of NEXTFLEX® RiboNaut™ Bead Wash Buffer. Scale up or down accordingly.

6. Repeat steps 3-5 for a total of two bead washes.
7. For one sample, resuspend the pellet in 45 µL of NEXTFLEX® RiboNaut™ Bead Wash Buffer. For 5 samples, resuspend the pellet in 225 µL of NEXTFLEX® RiboNaut™ Bead Wash Buffer. Scale up or down accordingly.
8. Proceed to Step B: Hybridize Baits to rRNA.

Note: Use beads immediately after preparation. Do not store.

Step B: Hybridize Baits to rRNA

MATERIALS

PINK CAP - NEXTFLEX® RiboNaut™ Bait Mix

PINK CAP - NEXTFLEX® Bait Hybridization Buffer

WHITE CAP - Nuclease-free water

User Supplied

- Total RNA (5 ng -1 µg) in nuclease-free water
- 96-well PCR plate
- 96-well PCR plate magnetic stand

Input RNA Quality:	RQS/RIN:	Input range:
Intact	10 – 7	5 – 1000 ng
Partially degraded	6.9 – 3	5 – 100 ng
Highly degraded or FFPE total RNA	< 3	5 – 50 ng

! NOTE: Using quantities of RNA greater than the maximum input recommendation will lead to carry-over of rRNA.

1. For each sample, combine the following reagents in a 96-well PCR plate

_ µL	Total RNA
_ µL	Nuclease-free water
4 µL	NEXTFLEX® Bait Hybridization Buffer
5 µL	NEXTFLEX® RiboNaut™ Bait Mix
20 µL	TOTAL

2. Mix thoroughly by pipetting and seal the 96-well PCR plate.
3. Program a thermal cycler and incubate as follows:

5 min	70°C
≥ 10 min	4°C

4. Incubate at room temperature for ≥ 10 minutes.
5. Proceed to Step C: Capture bait-hybridized RNA and purify rRNA depleted RNA.

Step C: Capture Bait-hybridized RNA & Purify rRNA-depleted RNA

MATERIALS

CLEAR CAP - NEXTFLEX® RiboNaut™ Beads

PINK CAP or CLEAR CAP BOTTLE - NEXTFLEX® Cleanup Buffer

BROWN CAP - NEXTFLEX® Cleanup Beads XP

User Supplied

- 20 µL bait-hybridized RNA (from Step B)
- Washed NEXTFLEX® RiboNaut™ Beads (from Step A)
- 96-well PCR plate
- 96-well PCR plate magnetic stand

1. In a new 96-well PCR plate, add 65 µL of washed NEXTFLEX® RiboNaut™ Beads from STEP A to each well.
2. Add 20 µL of bait-hybridized RNA sample to each well.
3. Incubate at room temperature for ≥ 10 minutes.
4. Place the 96-well PCR plate on the magnetic stand at room temperature for 30 seconds or until the supernatant appears completely clear.
5. In a new 96-well PCR plate, add 20 µL of NEXTFLEX® Cleanup Beads XP and 95 µL of NEXTFLEX® Cleanup Buffer for a total of 115 µL. Mix thoroughly by pipetting.
6. Transfer 60 µL of supernatant from STEP C, #4 to the 96-well PCR plate containing 115 µL of NEXTFLEX® Cleanup Beads XP and NEXTFLEX® Cleanup Buffer. Mix thoroughly by pipetting.
7. Incubate for 5 minutes.
8. Place plate on the magnetic stand for 5 minutes or until solution is clear.
9. Remove and discard the supernatant, taking care not to disturb the beads.
10. Add 200 µL of freshly prepared 80% ethanol, incubate for 30 seconds, and remove all of the supernatant. Repeat this step for a total of 2 ethanol washes.
    IMPORTANT: Always use freshly prepared 80% ethanol and do not incubate the bead pellet with 80% ethanol for extended periods.
11. Incubate sample for 3 minutes. After one minute, remove all residual liquid that may have collected at the bottom of the well.
12. Remove plate from magnetic stand and resuspend bead pellet in 17 µL of nuclease-free water by pipetting volume up and down. Ensure that the beads are completely resuspended.
13. Incubate sample for 2 minutes.
14. Place the plate on the magnetic stand for 3 minutes or until the supernatant appears clear.
15. Transfer 14 µL of supernatant to a new PCR plate. The rRNA-depleted total RNA is now ready for RNA-Seq based applications.
16. Store samples at -80 °C or proceed with Rapid Directional RNA-Seq Kit 2.0.

For total RNA inputs greater than 500 ng, electropherogram analysis using a LabChip® GXII Touch HT instrument or equivalent is recommended.

Representative rRNA-Depletion Profile

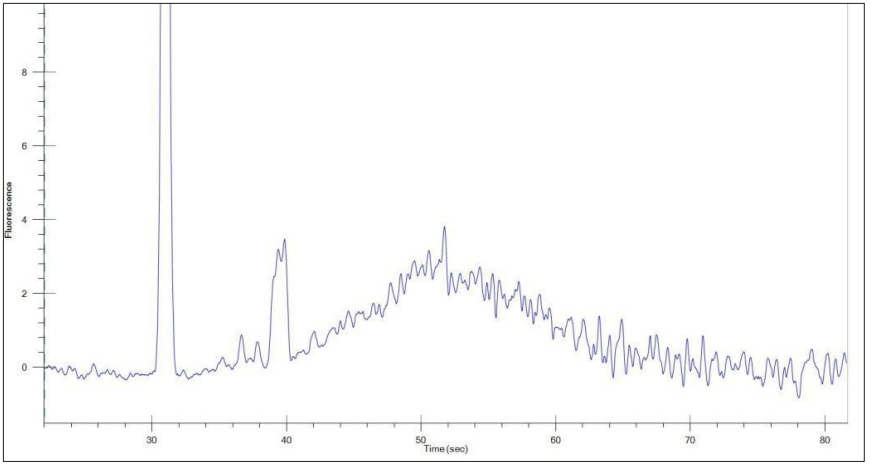


Figure 1: Example of 1 ug of Universal Human Reference RNA (Agilent® #740000) after rRNA depletion.

1 µL of rRNA-depleted RNA was run on the LabChip® GXII Touch™ HT instrument using the RNA Pico Assay Reagent Kit (# CLS960012) and a DNA/RNA/Charge Variant Assay LabChip (# 760435).

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