# Customized Depletion of Unwanted RNA

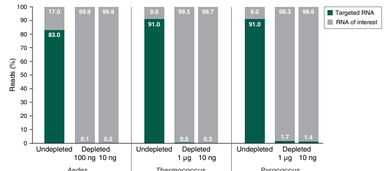
Are you working with a sample type for which current RNA depletion kits are not compatible, or do you need to remove a specific RNA from your sample? NEBNext now enables a customizable approach to deplete unwanted RNA from any organism, using probe sequences designed with a user-friendly web tool.

**STEP 1:** Use the online NEBNext Custom RNA Depletion Design Tool to obtain custom probe sequences, by entering the sequence of your target RNA.

**STEP 2:** Order ssDNA probe oligonucleotides from your trusted oligo provider.

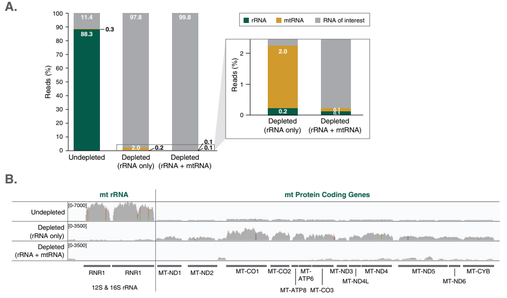
**STEP 3:** Use the probes with the NEBNext Custom RNA Depletion Core Reagent Set or in combination with other NEBNext RNA Depletion Kits.

**NEBNext Custom RNA Depletion enriches for RNAs of interest by efficiently removing targeted RNA from total RNA across species and a wide range of inputs**




*The NEBNext Custom RNA Depletion Design Tool was used to design probes against rRNA of the mosquito Aedes aegypti, and the archaeal species Thermococcus kodakarensis and Pyrococcus furiosus. Total RNA (1 µg, 100 ng or 10 ng) was used as input for rRNA depletion using the Core RNA Depletion Reagent Set with the designed probes. RNA-seq libraries were prepared using the NEBNext Ultra™ II Directional RNA Library Prep Kit for Illumina® followed by paired-end sequencing (2 x 75 bp). 20 million reads were sampled (seqtk) from each library. Read pairs were identified as ribosomal using mirabait (6 or more shared 25-mers), and levels of rRNA remaining were calculated by dividing matched reads by the total number of reads passing instrument quality filtering. The data represents an average of 3 replicates. The method efficiently depletes targeted rRNA across species and a wide range of total RNA input amount (1 µg–10 ng).*

**Combined probe pools efficiently deplete human rRNA and mitochondrial mRNA using NEBNext Custom RNA Depletion**




*The NEBNext Custom RNA Depletion Design Tool was used to design probes against human mitochondrial mRNA. The probes were used in combination with the probe pool from the NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat). 1 µg of total Universal Human Reference RNA (Agilent®) was depleted of mitochondrial RNA and rRNA using the Core RNA Depletion Reagent Set. RNA-seq libraries were prepared using the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina followed by paired-end sequencing (2 x 75 bp). 20 million reads were sampled (seqtk) from each library.*

*A. Read pairs were identified as ribosomal and mitochondrial using mirabait (6 or more, 25-mers), and levels of rRNA and mtRNA remaining were calculated by dividing matched reads by the total number of reads passing instrument quality filtering. Both rRNA and mitochondrial RNA are efficiently depleted.*

*B. Integrative Genome Viewer (IGV) visualization of read coverage across the human mitochondrial genes.*

# Ordering Information

| PRODUCT | NEB # | SIZE |
|---|---|---|
| NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) | E7400S/L/X | 6/24/96 rxns |
| NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) with RNA Sample Purification Beads | E7405S/L/X | 6/24/96 rxns |
| NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) | E7750S/L/X | 6/24/96 rxns |
| NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads | E7755S/L/X | 6/24/96 rxns |
| NEBNext rRNA Depletion Kit (Bacteria) | E7850S/L/X | 6/24/96 rxns |
| NEBNext rRNA Depletion Kit (Bacteria) with RNA Sample Purification Beads | E7860S/L/X | 6/24/96 rxns |
| NEBNext RNA Depletion Core Reagent Set | E7865S/L/X | 6/24/96 rxns |
| NEBNext RNA Depletion Core Reagent Set with RNA Sample Purification Beads | E7870S/L/X | 6/24/96 rxns |
| **COMPANION PRODUCTS** | | |
| Monarch® RNA Cleanup Kit (10 µg) | T2030S/L | 10/100 preps |
| NEBNext Poly(A) mRNA Magnetic Isolation Module | E7490S/L | 24/96 rxns |
| NEBNext Ultra II Directional RNA Library Prep Kit for Illumina | E7760S/L | 24/96 rxns |
| NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads | E7765S/L | 24/96 rxns |
| NEBNext Ultra II RNA Library Prep Kit for Illumina | E7770S/L | 24/96 rxns |
| NEBNext Ultra II RNA Library Prep with Sample Purification Beads | E7775S/L | 24/96 rxns |
| NEBNext Library Quant Kit for Illumina | E7630S/L | 100/500 rxns |
| NEBNext Magnetic Separation Rack | S1515S | 24 tubes |



Request a free sample at **NEBNext.com**

# Featured Online Tool

**NEBNext® Custom RNA Depletion Design Tool**

Design oligos for depletion of unwanted RNA from any organism, when used in the NEBNext RNA depletion workflow.
**https://depletion-design.neb.com/**


**USA**
New England Biolabs, Inc.
Telephone (978) 927-5054
Toll Free (USA Orders) 1-800-632-5227
Toll Free (USA Tech) 1-800-632-7799
Fax (978) 921-1350
info@neb.com
www.neb.com

**Australia**
New England Biolabs (Australia) PTY
Telephone: +61 (1800) 934218
info.au@neb.com

**Canada**
New England Biolabs, Ltd.
Toll Free: 1-800-387-1095
info.ca@neb.com

**China**
New England Biolabs (Beijing), Ltd.
Telephone: 010-82378265/82378266
info@neb-china.com

**France**
New England Biolabs France
Telephone: 0800 100 632
info.fr@neb.com

**Germany & Austria**
New England Biolabs GmbH
Free Call: 0800/246 5227 (Germany)
Free Call: 00800/246 52277 (Austria)
info.de@neb.com

**Japan**
New England Biolabs Japan, Inc.
Telephone: +81 (0)3 5669 6191
info.jp@neb.com

**Singapore**
New England Biolabs, PTE. Ltd.
Telephone: +65 638 59623
sales.sg@neb.com

**United Kingdom**
New England Biolabs (UK), Ltd.
Call Free: 0800 318486
info.uk@neb.com

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RNA_Depletion – Version 3.0 – 12/20


Now includes NEBNext RNA Depletion Core Reagent Set

# NEBNext® RNA Depletion

GET MORE OF WHAT YOU WANT

NEW ENGLAND BioLabs Inc.

*be* INSPIRED
*drive* DISCOVERY
*stay* GENUINE

# Get more of what you want...

## NEBNext RNA Depletion Kits

Abundant RNAs can conceal the biological significance of less-abundant transcripts, making their efficient and specific removal desirable. NEBNext RNA Depletion kits facilitate the removal of abundant RNAs, while ensuring retention of RNAs of interest. These kits employ the efficient RNase H method (1,2) and close probe coverage of the undesirable, abundant RNA species, thereby ensuring that even degraded RNA is efficiently removed.


## Highlights:

- Suitable for low-quality (e.g., FFPE) and high-quality RNA
- Compatible with a broad range of input amounts: 10 ng–1 µg
- Superior depletion of abundant RNAs, with retention of RNAs of interest
- Fast workflow: 2 hours, with less than 10 minutes hands-on time
- Depleted RNA is suitable for RNA-seq, random-primed cDNA synthesis, or other downstream RNA analysis applications
- Available with optional Agencourt® RNAClean® XP Beads for RNA Purification
- Customizable option to deplete unwanted RNA from any organism, using probe sequences designed with a user-friendly web tool


*References*
*1. Adiconis, X. et al. (2013). Nature Methods 10, 623-629.*
*2. Morlan, J.D. et al. (2012). PLoS One 7, e42847.*

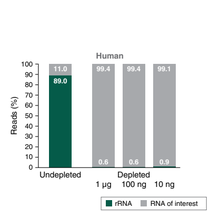

# From your Human, Mouse, Rat, Blood and Bacterial RNA Samples.

## For rRNA Depletion from Human, Mouse and Rat

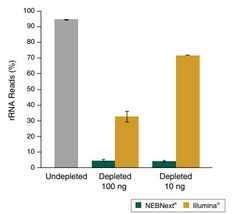
(NEB #E7400, #E7405)

Suitable for use with total RNA preparations from human, mouse and rat samples, these kits are optimized for depletion of both cytoplasmic (5S, 5.8S, 18S, 28S, ITS and ETS) and mitochondrial (12S, 16S) ribosomal RNA, from intact and degraded samples.

The NEBNext rRNA Depletion Kit v2 enriches for RNAs of interest across a wide range of total RNA inputs in human




The NEBNext rRNA Depletion Kit v2 efficiently depletes rRNA from degraded FFPE total RNA while preserving transcript abundances




*Universal human reference total RNA (A) or human adult normal liver tissue FFPE Total RNA, RIN 2.3 (B) was depleted of rRNA using the NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) (A and B), or the TruSeq® Stranded Total RNA Gold kit (B). RNA-seq libraries were prepared using NEBNext Ultra II Directional RNA Library Prep Kit for Illumina followed by paired-end sequencing (2 x 75 bp). 10 Million reads (A) or 20 Million reads from depleted libraries and 200 million reads from undepleted libraries (B) reads were sampled (seqtk) and were identified as ribosomal using mirabait.*

## For Depletion of Globin mRNA & rRNA for Human, Mouse and Rat

(NEB #E7750, #E7755)

In blood samples, the great majority of RNA comprises rRNA and globin mRNA, and their simultaneous removal is advantageous. The NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) depletes globin mRNA (HBA1/2, HBB, HBD, HBM, HBG1/2, HBE1, HBQ1 and HBZ), cytoplasmic rRNA (5S, 5.8S, 18S, 28S, ITS and ETS) and mitochondrial rRNA (12S, 16S). The kit is effective with human, mouse and rat total RNA preparations, both intact and degraded. When only mRNA (and not non-coding RNA) is of interest, the Globin & rRNA Depletion Kits can be used following poly(A) mRNA enrichment (e.g., using the NEBNext poly(A) mRNA Magnetic Isolation Module, NEB #E7490).

Depletion of globin mRNA and ribosomal RNA enriches for RNAs of interest across species


*Human, mouse and rat whole blood total RNA (1 µg) was depleted of rRNA alone, or rRNA and globin mRNA transcripts, using the NEBNext Globin & rRNA Depletion Kit. RNA-seq libraries were prepared from untreated and depleted RNA using the NEBNext Ultra II RNA Library Prep Kit for Illumina followed by paired-end sequencing (2 x 75 bp). Reads were identified as rRNA or globin mRNA using mirabait (6 or more, 25-mers), and levels of rRNA and globin mRNA remaining were calculated by dividing matched reads by the total number of reads passing instrument quality filtering.*

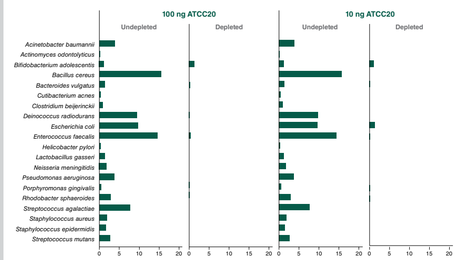
## For Depletion of Bacterial rRNA

(NEB #E7850, #E7860)

Specific enrichment of bacterial mRNAs is challenging due to their lack of poly(A) tails, precluding the use of oligo d(T)-based enrichment methods. For these samples, specific removal of bacterial rRNAs is an efficient way to enrich for RNAs of interest.

The NEBNext rRNA Depletion Kit (Bacteria) employs the NEBNext RNase H-based RNA depletion workflow to target removal of rRNA (5S, 16S and 23S) from gram-positive and gram-negative organisms. The method is effective with intact and degraded RNA, whether from monocultures or samples with mixed bacterial species.

Depletion of ribosomal RNA enriches for RNAs of interest, and maintains expression correlation, across a mock community of bacterial species and a range of input amounts




*Total RNA was extracted from a lyophilized pool of 20 different bacterial organisms (ATCC® #MSA-2002). Ribosomal RNA was depleted using the NEBNext rRNA Depletion Kit (Bacteria). RNA-seq libraries were prepared from untreated and depleted RNA using the NEBNext Ultra™ II Directional RNA Library Prep Kit for Illumina® followed by paired-end sequencing (2 x 75 bp). 4 Million read pairs were sampled (seqtk) from each library, mapped to a composite genome (Bowtie 2.3.2) before counting reads on genes (htseq-count) and correlating their levels. Effective depletion of sequences overlapping with annotated rRNA regions was observed at 100 ng and 10 ng of input RNA for most of the organisms. Correlation analysis of the transcripts indicates consistent transcript expression regardless of treatment or input amount.*

### What users are saying:

> *"NEB Bacterial Depletion has depleted rRNA equally or better than our previous ribodepletion gold standard across a wide RIN quality range. We have been pleased with the flexibility of Total RNA input ranges and have routinely gotten effective ribodepletion at 100 ng Total RNA Input in both single isolates and metagenomic samples. The protocol is also more ergonomically friendly than bead based ribodepletion protocols. Of all the new bacterial ribodepletion methods we have tested, NEB was by far the best."*
>
> Research Assistant,
> Biomedical Research Institution