



Now includes  
NEBNext RNA Depletion Core Reagent Set

# NEBNext<sup>®</sup> RNA Depletion

GET MORE OF WHAT YOU WANT

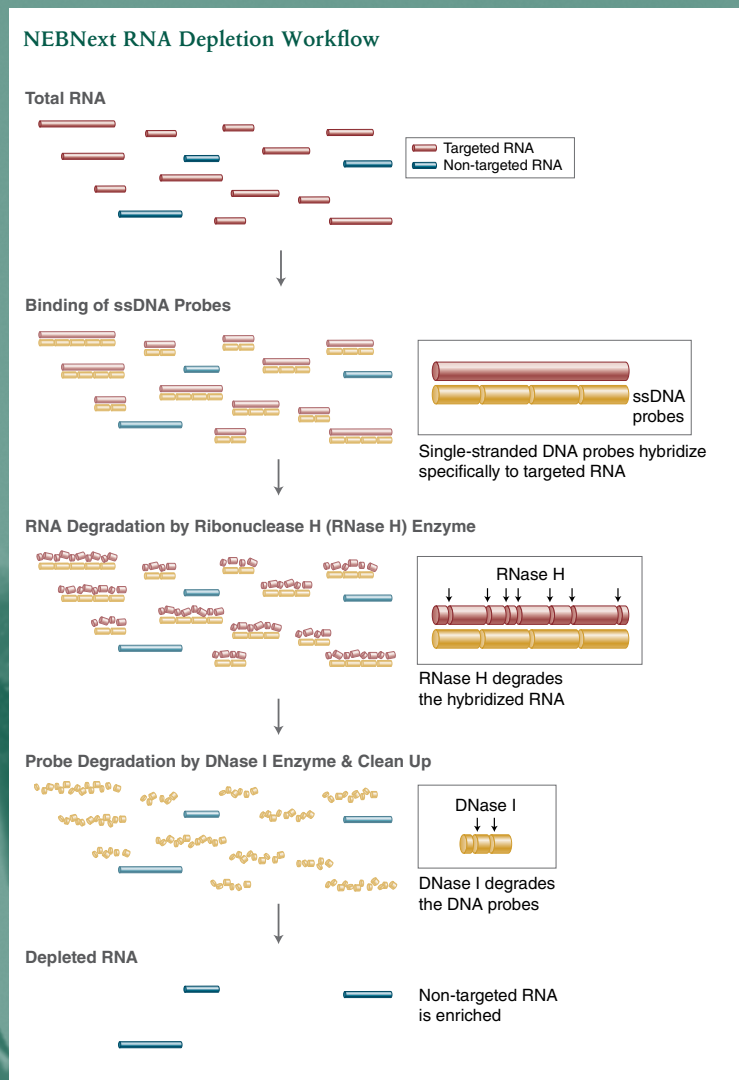




# Get more of what you want...

## 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, e42882.

# From your Human, Mouse, Rat, B

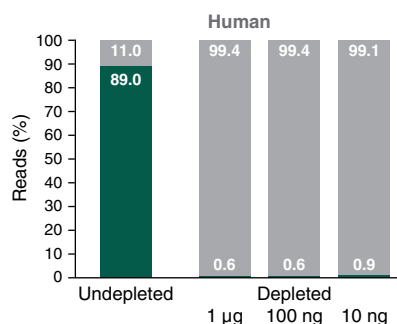
## For rRNA Depletion from Human, Mouse and Rat

(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.

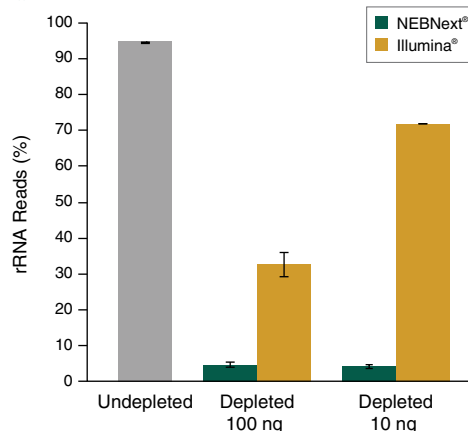
The NEBNext rRNA Depletion Kit v2 enriches for RNAs of interest across a wide range of total RNA inputs in human

A.



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

B.



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.

# Blood and Bacterial RNA Samples.

## 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.

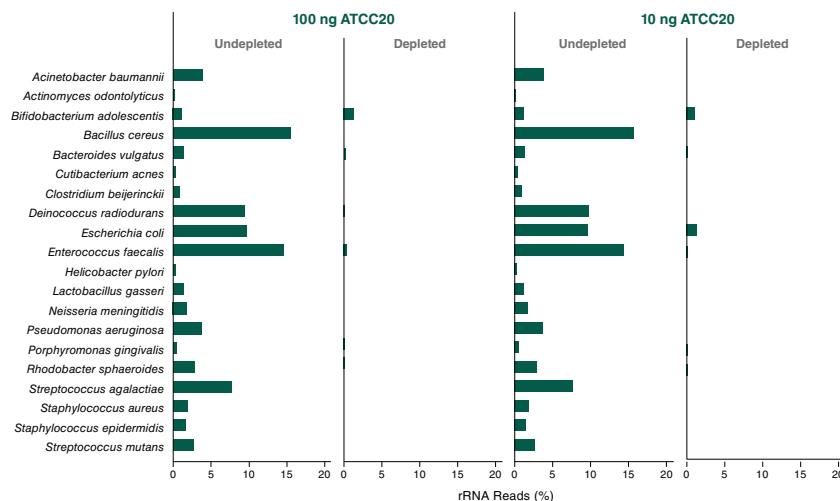
The NEBNext rRNA Depletion Kit (Bacteria) employs the NEBNext RNase H-based RNA depletion workflow to target removal of rRNA (5S, 16S and 23S) from gram-positive and gram-negative organisms. The method is effective with intact and degraded RNA, whether from monocultures or samples with mixed bacterial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

### 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

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.



# Customized Depletion of Unwanted RNA

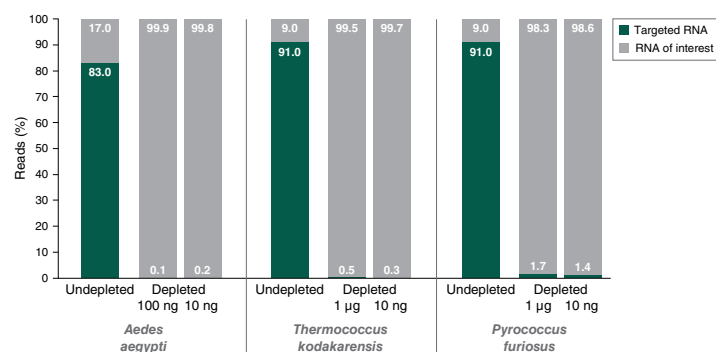
Are you working with a sample type for which current RNA depletion kits are not compatible, or do you need to remove a specific RNA from your sample? NEBNext now enables a customizable approach to deplete unwanted RNA from any organism, using probe sequences designed with a user-friendly web tool.

**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.

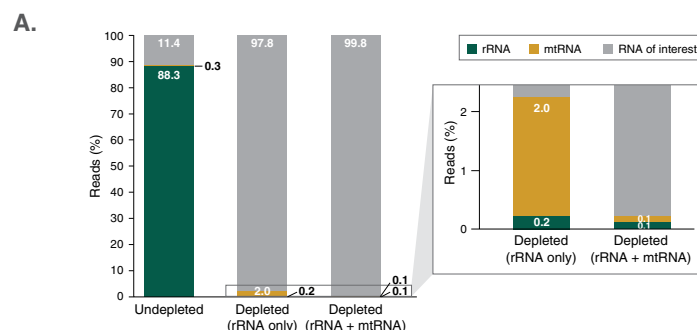
**STEP 3:** Use the probes with the NEBNext Custom RNA Depletion Core Reagent Set or in combination with other NEBNext RNA Depletion Kits.

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)	<a href="#">E7400S/L/X</a>	6/24/96 rxns
NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) with RNA Sample Purification Beads	<a href="#">E7405S/L/X</a>	6/24/96 rxns
NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat)	<a href="#">E7750S/L/X</a>	6/24/96 rxns
NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	<a href="#">E7755S/L/X</a>	6/24/96 rxns
NEBNext rRNA Depletion Kit (Bacteria)	<a href="#">E7850S/L/X</a>	6/24/96 rxns
NEBNext rRNA Depletion Kit (Bacteria) with RNA Sample Purification Beads	<a href="#">E7860S/L/X</a>	6/24/96 rxns
NEBNext RNA Depletion Core Reagent Set	<a href="#">E7865S/L/X</a>	6/24/96 rxns
NEBNext RNA Depletion Core Reagent Set with RNA Sample Purification Beads	<a href="#">E7870S/L/X</a>	6/24/96 rxns
COMPANION PRODUCTS		
Monarch® RNA Cleanup Kit (10 µg)	<a href="#">T2030S/L</a>	10/100 preps
NEBNext Poly(A) mRNA Magnetic Isolation Module	<a href="#">E7490S/L</a>	24/96 rxns
NEBNext High Input Poly(A) mRNA Isolation Module	<a href="#">E3370S</a>	24 rxns
NEBNext UltraExpress RNA Library Prep Kit	<a href="#">E3330S/L</a>	24/96 rxns
NEBNext Ultra II Directional RNA Library Prep Kit for Illumina	<a href="#">E7760S/L</a>	24/96 rxns
NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads	<a href="#">E7765S/L</a>	24/96 rxns
NEBNext Ultra II RNA Library Prep Kit for Illumina	<a href="#">E7770S/L</a>	24/96 rxns
NEBNext Ultra II RNA Library Prep with Sample Purification Beads	<a href="#">E7775S/L</a>	24/96 rxns
NEBNext Library Quant Kit for Illumina	<a href="#">E7630S/L</a>	100/500 rxns
NEBNext Magnetic Separation Rack	<a href="#">S1515S</a>	24 tubes

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[NEBNext.com](http://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  
[info@neb.com](mailto:info@neb.com)

### Australia & New Zealand

New England Biolabs (Australia) PTY  
Telephone: 1800 934 218 (AU)  
[info.au@neb.com](mailto:info.au@neb.com)  
Telephone: 0800 437 209 (NZ)  
[info.nz@neb.com](mailto:info.nz@neb.com)

### Canada

New England Biolabs, Ltd.  
Toll Free: 1-800-387-1095  
[info.ca@neb.com](mailto:info.ca@neb.com)

### China

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

### France

New England Biolabs France  
Telephone: 0800 100 632  
[info.fr@neb.com](mailto: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](mailto:info.de@neb.com)

### Japan

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

### Singapore

New England Biolabs, Pte. Ltd.  
Telephone: +65 638 59623  
[sales.sg@neb.com](mailto:sales.sg@neb.com)

### Republic of Korea

New England Biolabs Korea Ltd.  
Toll Free: +82 (70) 47318478  
[info.kr@neb.com](mailto:info.kr@neb.com)

### United Kingdom

New England Biolabs (UK), Ltd.  
Call Free: 0800 318486  
[info.uk@neb.com](mailto:info.uk@neb.com)

[www.neb.com](http://www.neb.com)



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