

# NEBNext<sup>®</sup> RNA Depletion

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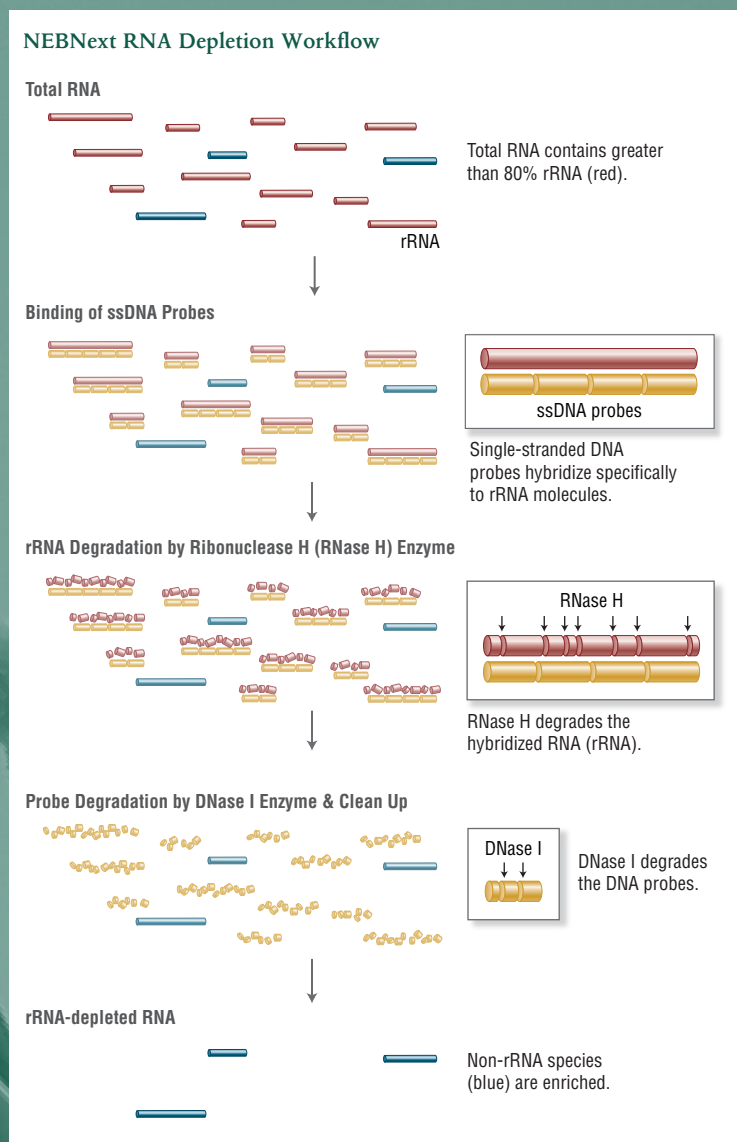
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# Get more of what you want...

## 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 tiling 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

### References

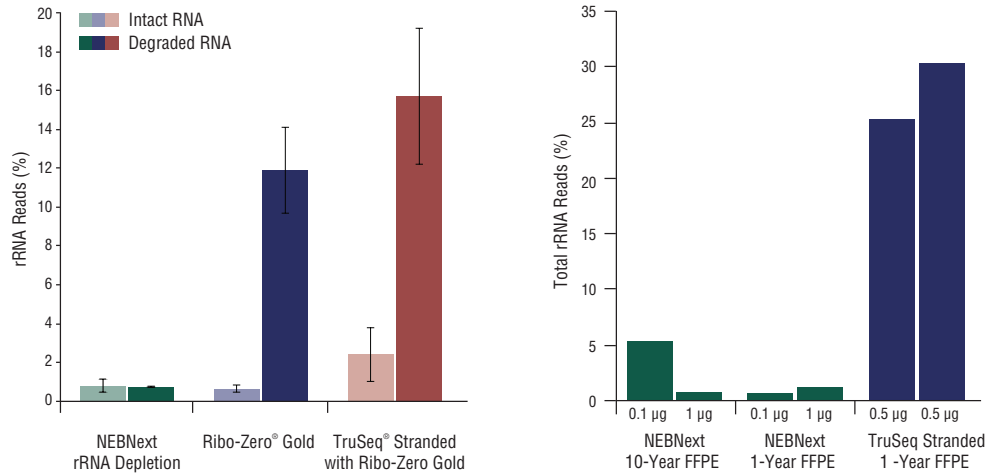
1. Adiconis, X. et al. (2013). *Nature Methods* 10: 623-629.
2. Morlan, J.D. et al. (2012). *PLoS One* 7, e42882.

# From your Human, Mouse, Rat, B

## For rRNA Depletion from Human, Mouse and Rat

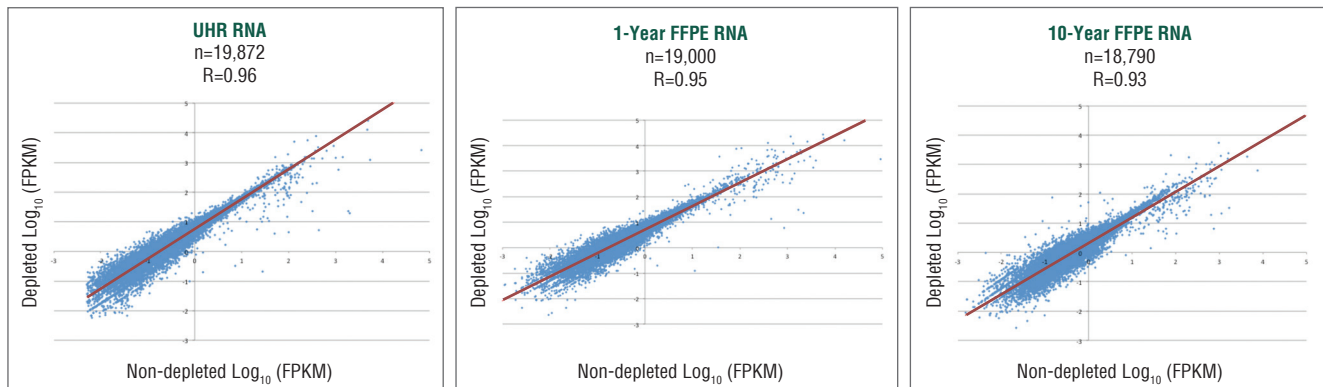
Suitable for use with total RNA preparations from human, mouse and rat samples, these kits are optimized for depletion of both cytoplasmic (5S, 5.8S, 18S and 28S) and mitochondrial (12S, 16S) ribosomal RNA, from intact and degraded samples.

### rRNA depletion efficiency with intact and degraded RNA



Ribosomal RNA was depleted from (A) intact Universal Human Reference Total RNA (UHR, Agilent) (RIN > 9) and degraded UHR Total RNA (RIN < 3) and (B) 0.1 µg, 0.5 µg or 1 µg of breast cancer FFPE RNA samples (with archive ages of one year and 10 years) using either the NEBNext rRNA Depletion Kit (Human/Mouse/Rat), or the TruSeq® Stranded Total RNA Kit with Ribo-Zero Gold. rRNA-depleted RNA libraries were made using either the NEBNext Ultra Directional RNA Library Prep Kit for Illumina or the TruSeq Stranded Total RNA Kit with Ribo-Zero Gold. Total rRNA-aligned reads were determined using Bowtie 2.0 (local, sensitive). NEBNext rRNA-depleted libraries contain a minimal percentage of rRNA reads regardless of the quality of the RNA.

### Transcript expression correlation with non-depleted libraries



Libraries were made from UHR RNA (Agilent) and Breast Cancer FFPE RNA (with archive age of one year and 10 years), both non-depleted and depleted for rRNA using the NEBNext rRNA Depletion Kit (Human/Mouse/Rat). All libraries were made using the NEBNext Ultra Directional RNA Library Prep Kit for Illumina. TopHat2 and Cufflinks were used for read mapping and transcript assembly and quantification. FPKM (Fragments Per Kilobase of transcript per Million mapped reads) correlation analysis indicates very good transcript expression correlation ( $R > 0.93$ ) between Depleted and Non-Depleted libraries. NEBNext rRNA depletion does not affect transcript expression levels.

# Blood and Bacterial RNA Samples.

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

The NEBNext RNase H-based depletion method can be applied to abundant RNAs beyond rRNA. 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).

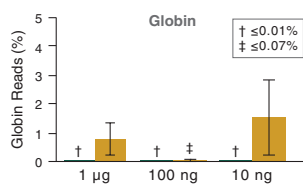
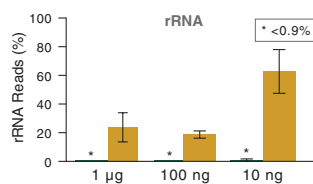
### Depletion of globin mRNA and ribosomal RNA enriches for RNAs of interest across species



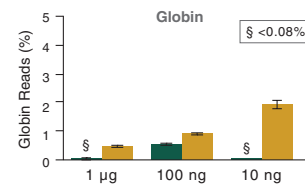
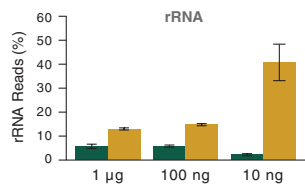
Human, mouse and rat whole blood total RNA (1  $\mu$ g) was depleted of rRNA alone, or rRNA and globin mRNA transcripts, using the NEBNext Globin & rRNA Depletion Kit. RNA-seq libraries were prepared from untreated and depleted RNA using the NEBNext Ultra II RNA Library Prep Kit for Illumina followed 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.

### Consistent depletion of globin mRNA and rRNA across species and across inputs

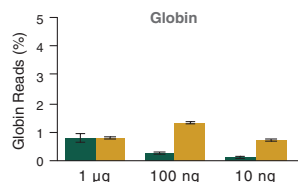
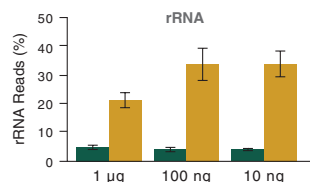
#### A. Human Whole Blood



#### B. Mouse Whole Blood



#### C. Rat Whole Blood



Human, mouse and rat whole blood total RNA (1  $\mu$ g, 100 ng and 10 ng) was depleted of rRNA and globin mRNA using the NEBNext Globin & rRNA Depletion Kit or Globin-Zero<sup>®</sup> Gold rRNA Depletion Kit (Illumina). 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 ribosomal or globin 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. The data represents an average of 3 replicates and error bars indicate standard error. The NEBNext Globin Depletion Kit is superior at depleting rRNA across species, and at depleting over 99% of globin mRNA.

# For Depletion of Bacterial rRNA

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.

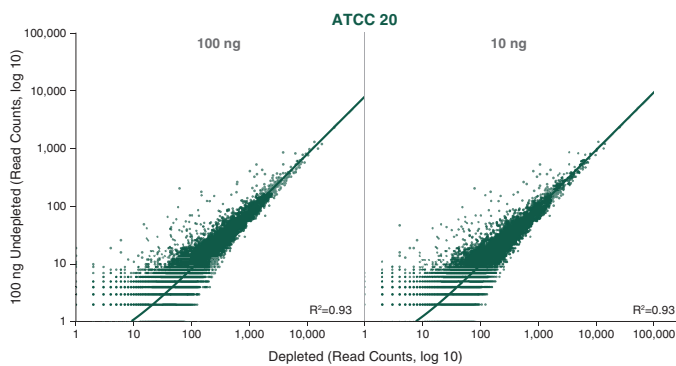
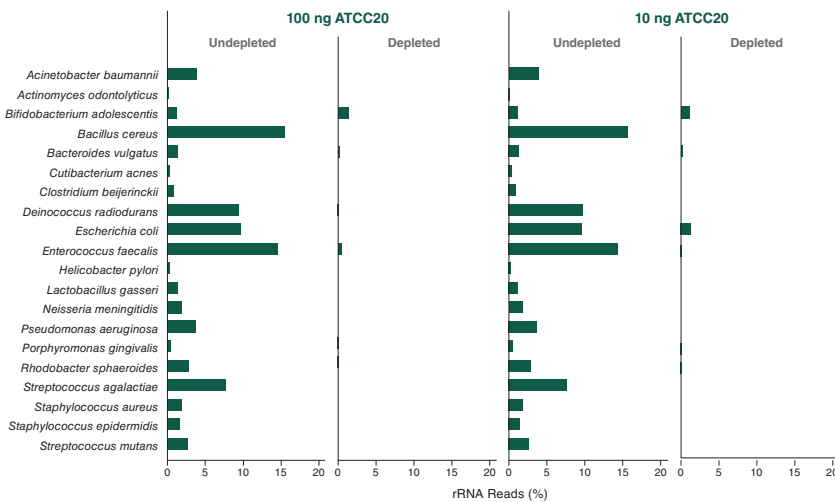
The NEBNext rRNA Depletion Kit (Bacteria) employs the NEBNext RNase H-based RNA depletion workflow to target removal of rRNA (5S, 16S and 23S) from gram-positive and gram-negative organisms. The method is effective with intact and degraded RNA, whether from monocultures or samples with mixed bacterial 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. 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 × 75 bp). A. Reads were aligned (Hisat 2) to a composite reference genome containing the best matching strains in the NCBI genome database. Alignments were duplicate marked (Picard) and assessed for transcript levels (ht-seq count). B. 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.

# Ordering Information

| PRODUCT  | VWR CAT. NO. | SIZE          |
|--|--------------|---------------|
| NEBNext rRNA Depletion Kit (Human/Mouse/Rat)   | 102877-568   | 6 reactions   |
|  | 102877-566   | 24 reactions  |
|  | 102877-570   | 96 reactions  |
| NEBNext rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads          | 103501-482   | 6 reactions   |
|  | 103501-480   | 24 reactions  |
|  | 103501-484   | 96 reactions  |
| NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat)                                    | 76377-490    | 6 reactions   |
|  | 76377-488    | 24 reactions  |
|  | 76377-492    | 96 reactions  |
| NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads | 76377-496    | 6 reactions   |
|  | 76377-494    | 24 reactions  |
|  | 76377-498    | 96 reactions  |
| NEBNext rRNA Depletion Kit (Bacteria)  | 76407-334    | 6 reactions   |
|  | 76407-332    | 24 reactions  |
|  | 76407-336    | 96 reactions  |
| NEBNext rRNA Depletion Kit (Bacteria) with RNA Sample Purification Beads                 | 76407-340    | 6 reactions   |
|  | 76407-338    | 24 reactions  |
|  | 76407-342    | 96 reactions  |
| <b>COMPANION PRODUCTS</b>  |              |               |
| Monarch® RNA Cleanup Kit (10 µg)   | 76307-360    | 10 preps      |
|  | 76307-460    | 100 preps     |
| NEBNext Poly(A) mRNA Magnetic Isolation Module   | 102715-962   | 24 reactions  |
|  | 102715-960   | 96 reactions  |
| NEBNext Ultra II Directional RNA Library Prep Kit for Illumina                           | 103501-464   | 24 reactions  |
|  | 103501-462   | 96 reactions  |
| NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads             | 103501-468   | 24 reactions  |
|  | 103501-466   | 96 reactions  |
| NEBNext Ultra II RNA Library Prep Kit for Illumina                                       | 103501-446   | 24 reactions  |
|  | 103501-444   | 96 reactions  |
| NEBNext Ultra II RNA Library Prep with Sample Purification Beads                         | 103501-456   | 24 reactions  |
|  | 103501-454   | 96 reactions  |
| NEBNext Library Quant Kit for Illumina   | 102942-190   | 100 reactions |
|  | 102942-192   | 500 reactions |
| NEBNext Magnetic Separation Rack   | 76326-058    | 24 tubes      |



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