# NEBNext rRNA Depletion Kit (Human/Mouse/Rat) E6310

**New England Biolabs**

## Abstract

The NEBNext rRNA Depletion Kit (Human/Mouse/Rat) depletes both cytoplasmic (5S rRNA, 5.8S rRNA, 18S rRNA and 28S rRNA) and mitochodrial ribosomal RNA (12S rRNA and 16S rRNA) from human, mouse and rat total RNA preparations. This product is suitable for both intact and degraded RNA (e.g. FFPE RNA). The resulting rRNA-depleted RNA is suitable for RNA-Seq, random-primed cDNA synthesis, or other downstream RNA analysis applications.

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## Before start

**Starting Material:** 5 ng–1 µg total RNA (DNA free) in a 12 µl total volume. If the total RNA may contain gDNA contamination, treat the RNA sample with DNase I to remove all traces of DNA, then purify the treated RNA to remove DNase I.

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### RNA Sample Requirements

The RNA sample should be free of salts (e.g., Mg2+, or guanidinium salts) or organics (e.g., phenol and ethanol). RNA must be free of DNA. gDNA is a common contaminant from RNA preps. It may be carried over from the interphase of organic extractions or when the silica matrix of solid phase RNA purification methods is overloaded. If the total RNA sample may contain gDNA contamination, treat the sample with DNase I to remove all traces of DNA (not provided in this kit). After treatment with DNase I the enzyme should be removed from the sample. Any residual activity of DNase I will degrade the single stranded DNA probes necessary for the ribosomal depletion. DNase I can be removed from the extraction using phenol/chloroform extraction and ethanol precipitation.

### Typical Yield of rRNA-depleted RNA from a Reaction

The actual yield is dependent on the quality of the input RNA, the rRNA content of the sample, and the method used to purify the rRNA-depleted RNA. Recoveries of 3%–10% of the input RNA are typical.

### RNA Input

5 ng to 1 µg DNA free total RNA in up to 12 µl total volume.

Note: for RNAseq samples we recommend using total RNA inputs higher than 5 ng to increase library complexity and reduce sequencing duplication rates.

When using NEBNext rRNA Depletion Kit (Human/Mouse/Rat; NEB #E6310 or #E6350) with the below NEBNext kits please follow the appropriate chapter in the corresponding kit manual and start with the appropriate input amount of RNA.

NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB #E7760)

NEBNext Ultra II RNA Library Prep Kit for Illumina (NEB #E7770)

NEBNext Ultra Directional RNA Library Prep Kit for Illumina (NEB #E7420)

NEBNext Ultra RNA Library Prep Kit for Illumina (NEB #E7530)

## Materials

- NEBNext RNase H E6318 by New England Biolabs
- RNase H Reaction Buffer E6312 by New England Biolabs
- NEBNext rRNA Depletion Solution E6313 by New England Biolabs
- NEBNext Probe Hybridization Buffer E6314 by New England Biolabs
- DNase I (RNase-free) E6316 by New England Biolabs
- DNase I Reaction Buffer E6315 by New England Biolabs
- Nuclease-free Water E6317 by New England Biolabs
- NEBNext RNA Sample Purification Beads E6315 by New England Biolabs
- Magnetic Rack View by Contributed by users
- 80% Ethanol (freshly prepared) View by Contributed by users
- Thermal cycler View by Contributed by users
- Agencourt RNAClean XP Beads A63987 by Beckman Coulter
- DNase I (e.g., NEB #M0303) and DNase I Cleanup Reagants or Kit for Removal of DNA Prior to Depletion View by New England Biolabs

## Protocol

### Hybridize the Probes to the RNA

***Step 1.***

Prepare a RNA/Probe master mix as follows:

| Component | Volume |
|---|---|
| NEBNext rRNA Depletion Solution | 1 µl |

| | |
|---|---|
| Probe Hybridization Buffer | 2 µl |
| Total Volume | 3 µl |

REAGENTS

- NEBNext rRNA Depletion Solution E6313 by New England Biolabs
- NEBNext Probe Hybridization Buffer E6314 by New England Biolabs

## Hybridize the Probes to the RNA

***Step 2.***

Add 3 µl of the above mix to 12 µl total RNA sample.

## Hybridize the Probes to the RNA

***Step 3.***

Mix by pipetting up and down at least 10 times.

## Hybridize the Probes to the RNA

***Step 4.***

Spin down briefly in a tabletop centrifuge, and immediately proceed to the next step.

## Hybridize the Probes to the RNA

***Step 5.***

Place samples in a thermocycler with a heated lid set to approximately 105°C, and run the following program, which will take approximately 15–20 minutes to complete:

| Temp | Time |
|---|---|
| 95°C | 2 min |
| 95-22°C | 0.1°C/sec |
| 22°C | 5 min hold |

## Hybridize the Probes to the RNA

***Step 6.***

Spin down the samples in a tabletop centrifuge, and place on ice. Proceed immediately to the next step.

## RNase H Digestion

***Step 7.***

On ice, prepare a master mix according to the following table, and mix by pipetting up and down at least 10 times; use immediately.

| Component | Volume |
|---|---|
| NEBNext RNase H | 2 µl |
| RNase H Reaction Buffer | 2 µl |

| | |
|---|---|
| Nuclease-free Water | 1 µl |
| Total Volume | 5 µl |

REAGENTS

- NEBNext RNase H E6318 by New England Biolabs
- RNase H Reaction Buffer E6312 by New England Biolabs
- Nuclease-free Water E6317 by New England Biolabs

RNase H Digestion

***Step 8.***

Add 5 µl of the above mix to the RNA sample from Step 6.

RNase H Digestion

***Step 9.***

Mix by pipetting up and down at least 10 times.

RNase H Digestion

***Step 10.***

Spindown briefly in a table top centrifuge and immediately proceed to the next step.

RNase H Digestion

***Step 11.***

Place samples in a thermocycler (with lid at 40°C or off) and incubate at 37°C for 30 minutes.

DURATION

00:30:00 : Incubate

RNase H Digestion

***Step 12.***

Spin down the samples in a tabletop centrifuge, and place on ice. Proceed immediately to the next step to prevent non-specific degradation of RNA.

DNase I Digestion

***Step 13.***

On ice, prepare a DNase I Digestion Master Mix according to the following table, and mix by pipetting up and down at least 10 times; use immediately.

| Component | Volume |
|---|---|
| DNase I Reaction Buffer | 5 µl |
| DNase I (RNase-free) | 2.5µl |
| Nuclease-free Water | 22.5µl |
| Total Volume | 30 µl |

REAGENTS

- DNase I Reaction Buffer E6315 by New England Biolabs
- DNase I (RNase-free) E6316 by New England Biolabs
- Nuclease-free Water E6317 by New England Biolabs

DNase I Digestion

***Step 14.***

Add 5 µl of the above mix to the RNA sample from Step 12.

DNase I Digestion

***Step 15.***

Mix by pipetting up and down at least 10 times.

DNase I Digestion

***Step 16.***

Spin down briefly in a table top centrifuge and immediately proceed to the next step.

DNase I Digestion

***Step 17.***

Place samples in a thermocycler (with lid at 40°C or off) and incubate at 37°C for 30 minutes.

DURATION

00:30:00 : Incubate

DNase I Digestion

***Step 18.***

Spin down the samples in a tabletop centrifuge, and place on ice. Proceed immediately to the next step.

RNA Purification after rRNA Depletion Using Agencourt RNAClean XP Beads or NEBNext RNA Sample Purification Beads

***Step 19.***

Vortex Agencourt RNAClean XP Beads or NEBNext RNA Sample Purification Beads to resuspend.

RNA Purification after rRNA Depletion Using Agencourt RNAClean XP Beads or NEBNext RNA Sample Purification Beads

***Step 20.***

Add 110 µl (2.2X) resuspended beads to the RNA Sample. Mix well by pipetting up and down at least 10 times. Be careful to expel all of the liquid out of the tip during the last mix. If centrifuging samples after mixing, be sure to stop the centrifugation before the beads start to settle out.

RNA Purification after rRNA Depletion Using Agencourt RNAClean XP Beads or NEBNext RNA Sample Purification Beads

***Step 21.***

Incubate samples on ice for 15 minutes.

DURATION
00:15:00 : Incubate

RNA Purification after rRNA Depletion Using Agencourt RNAClean XP Beads or NEBNext RNA Sample Purification Beads

***Step 22.***

Place the tube/plate on an appropriate magnetic stand to separate the beads from the supernatant. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing on the magnetic stand.

RNA Purification after rRNA Depletion Using Agencourt RNAClean XP Beads or NEBNext RNA Sample Purification Beads

***Step 23.***

After 5 minutes (or when the solution is clear), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain the RNA

**Caution: Do not discard the beads**.

RNA Purification after rRNA Depletion Using Agencourt RNAClean XP Beads or NEBNext RNA Sample Purification Beads

***Step 24.***

Add 200 µl of 80% freshly prepared ethanol to the tube/ plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain the RNA.

DURATION
00:00:30 : Incubate

RNA Purification after rRNA Depletion Using Agencourt RNAClean XP Beads or NEBNext RNA Sample Purification Beads

***Step 25.***

Repeat Step 24 once for a total of two washes. Be sure to remove all visible liquid after the second wash. If necessary, briefly spin the tube/plate, place back on the magnet and remove traces of ethanol with a p10 pipette tip.

GOTO
Repeat -> go to step #24

RNA Purification after rRNA Depletion Using Agencourt RNAClean XP Beads or NEBNext RNA Sample Purification Beads

***Step 26.***

Air dry the beads for up to 5 minutes while the tube/plate is on the magnetic stand with the lid open.

**Caution: Do not over-dry the beads. This may result in lower recovery of RNA target. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack they are too dry.**

RNA Purification after rRNA Depletion Using Agencourt RNAClean XP Beads or NEBNext RNA Sample Purification Beads

***Step 27.***

Remove the tube/plate from the magnetic stand. Elute the RNA from the beads by adding 8 µl of nuclease free water.

REAGENTS

Nuclease-free Water E6317 by New England Biolabs

RNA Purification after rRNA Depletion Using Agencourt RNAClean XP Beads or NEBNext RNA Sample Purification Beads

***Step 28.***

Mix well by pipetting up and down 10 times. Incubate for at least 2 minutes. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing back on the magnetic stand.

RNA Purification after rRNA Depletion Using Agencourt RNAClean XP Beads or NEBNext RNA Sample Purification Beads

***Step 29.***

Place the tube/plate on the magnetic stand. After 5 minutes (or when the solution is clear), transfer 6 µl to a new PCR tube.

RNA Purification after rRNA Depletion Using Agencourt RNAClean XP Beads or NEBNext RNA Sample Purification Beads

***Step 30.***

Place the tube on ice and proceed with NGS library construction or other downstream application. Alternatively, the sample can be stored at –80°C.

TEMPERATURE

-80 °C : Storing Temp (if storing)

NOTES

**Recommended:**

To make sure rRNA is efficiently depleted, design RT aqPCR primers for the sample species rRNA and primers for a housekeeping gene. Compare rRNA content before and after ribosomal depletion to assess the rRNA removal efficiency.