NEBNext® rRNA Depletion Kit (Human/Mouse/Rat)

NEB #E6310S/L/X, E6350S/L/X

6/24/96 reactions

Version 6.0_2/20

Table of Contents

The Kit Includes

The volumes provided are sufficient for preparation of up to 6 reactions (NEB #E6310S/#E6350S), 24 reactions (NEB #E6310L/#E6350L) and 96 reactions (NEB #E6310X/#E6350X).

Package 1: Store at –20°C.

NEBNext RNase H

RNase H Reaction Buffer (10X)

NEBNext rRNA Depletion Solution

NEBNext Probe Hybridization Buffer

DNase I (RNase-free)

DNase I Reaction Buffer

Nuclease-free Water

Package 2: Store at 4°C. Do not freeze.

Supplied only with NEBNext rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads, NEB #E6350.

NEBNext RNA Sample Purification Beads

Required Materials Not Included

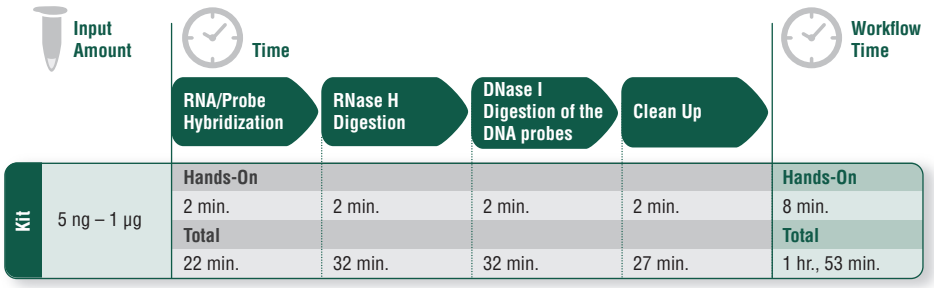
- Magnetic rack or plate (e.g., NEBNext Magnetic Separation Rack (NEB #S1515S), Alpaqua® 96S Super Magnet Plate (#A001322), or equivalent)
- 80% Ethanol (freshly prepared)
- Thermal Cycler
- DNase I (for example NEB #M0303) and DNase I cleanup reagents or kit

For NEB #E6310 only:

- Agencourt® RNAClean® XP Beads (Beckman Coulter, Inc. #A63987)

Applications

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.



RNA Sample Recommendations

The RNA sample should be free of salts (e.g., Mg^{2+}, 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)

Protocol for NEBNext rRNA Depletion Kit

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.

1. Hybridize the Probes to the RNA

1.1. 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

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

1.3. Mix by pipetting up and down at least 10 times.

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

1.5. 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

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

2. RNase H Digestion

2.1. 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

2.2. Add 5 µl of the above mix to the RNA sample from Step 1.6.

2.3. Mix by pipetting up and down at least 10 times.

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

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

2.6. 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.

3. DNase I Digestion

3.1. 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)	2.5 µl
Nuclease-free Water	22.5 µl
Total Volume	30 µl

3.2. Add 30 µl of the above mix to the RNA sample from Step 2.6.

3.3. Mix by pipetting up and down at least 10 times.

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

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

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

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

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

4.2. 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.

4.3. Incubate samples on ice for 15 minutes.

4.4. 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.

4.5. 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)**.

4.6. 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.

4.7. Repeat Step 4.6 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.

4.8. 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.

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

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.

4.10. 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.

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

Recommended: To make sure rRNA is efficiently depleted, design RT qPCR 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.

Please read the FAQ section on NEB.com for additional information about this product.

Kit Components

NEB #E6310S Table of Components

NEB #	PRODUCT	VOLUME
E6318-2	NEBNext RNase H	0.012 ml
E6312-2	RNase H Reaction Buffer	0.012 ml
E6313-2	NEBNext rRNA Depletion Solution	0.010 ml
E6314-2	NEBNext Probe Hybridization Buffer	0.012 ml
E6316-2	DNase I (RNase-free)	0.015 ml
E6315-2	DNase I Reaction Buffer	0.03 ml
E6317-2	Nuclease-free Water	0.4 ml

NEB #E6310L Table of Components

NEB #	PRODUCT	VOLUME
E6318-3	NEBNext RNase H	0.048 ml
E6312-3	RNase H Reaction Buffer	0.048 ml
E6313-3	NEBNext rRNA Depletion Solution	0.024 ml
E6314-3	NEBNext Probe Hybridization Buffer	0.048 ml
E6316-3	DNase I (RNase-free)	0.06 ml
E6315-3	DNase I Reaction Buffer	0.120 ml
E6317-3	Nuclease-free Water	1.5 ml

NEB #E6310X Table of Components

NEB #	PRODUCT	VOLUME
E6318-4	NEBNext RNase H	0.192 ml
E6312-4	RNase H Reaction Buffer	0.192 ml
E6313-4	NEBNext rRNA Depletion Solution	0.096 ml
E6314-4	NEBNext Probe Hybridization Buffer	0.192 ml
E6316-4	DNase I (RNase-free)	0.24 ml
E6315-4	DNase I Reaction Buffer	0.48 ml
E6317-4	Nuclease-free Water	6.0 ml

NEB #E6350S Table of Components

NEB #	PRODUCT	VOLUME
E6318-2	NEBNext RNase H	0.012 ml
E6312-2	RNase H Reaction Buffer	0.012 ml
E6313-2	NEBNext rRNA Depletion Solution	0.010 ml
E6314-2	NEBNext Probe Hybridization Buffer	0.012 ml
E6316-2	DNase I (RNase-free)	0.015 ml
E6315-2	DNase I Reaction Buffer	0.03 ml
E6317-2	Nuclease-free Water	0.4 ml
E6351S	NEBNext RNA Sample Purification Beads	0.66 ml

NEB #E6350L Table of Components

NEB #	PRODUCT	VOLUME
E6318-3	NEBNext RNase H	0.048 ml
E6312-3	RNase H Reaction Buffer	0.048 ml
E6313-3	NEBNext rRNA Depletion Solution	0.024 ml
E6314-3	NEBNext Probe Hybridization Buffer	0.048 ml
E6316-3	DNase I (RNase-free)	0.06 ml
E6315-3	DNase I Reaction Buffer	0.120 ml
E6317-3	Nuclease-free Water	1.5 ml
E6351L	NEBNext RNA Sample Purification Beads	2.64 ml

NEB #E6350X Table of Components

NEB #	PRODUCT	VOLUME
E6318-4	NEBNext RNase H	0.192 ml
E6312-4	RNase H Reaction Buffer	0.192 ml
E6313-4	NEBNext rRNA Depletion Solution	0.096 ml
E6314-4	NEBNext Probe Hybridization Buffer	0.192 ml
E6316-4	DNase I (RNase-free)	0.24 ml
E6315-4	DNase I Reaction Buffer	0.48 ml
E6317-4	Nuclease-free Water	6.0 ml
E6351X	NEBNext RNA Sample Purification Beads	10.6 ml

CheckList

1. Hybridize the Probes to the RNA

[_] 1.1. Prepare Master Mix

[_] 1.1.1. NEBNext rRNA Depletion Solution 1 µl

[_] 1.1.2. Probe Hybridization Buffer 2 µl

[_] 1.2. Add 3 µl of mix to 12 µl total RNA

[_] 1.3. Mix 10 times

[_] 1.4. Quick Spin

[_] 1.5. Put in Thermal cycler (95°C for 2 min, 95-22°C 0.1°C/sec, 22°C 5 min)

[_] 1.6. Quick spin, place on ice

2. RNase H Digestion

[_] 2.1. Prepare Master Mix and mix

[_] 2.1.1. RNase H 2 µl

[_] 2.1.2. RNase H Reaction Buffer 2 µl

[_] 2.1.3. Nuclease-free water 1 µl

[_] 2.2. Add 5 µl of master mix to sample

[_] 2.3. Mix 10 times

[_] 2.4. Quick Spin

[_] 2.5. Put in Thermal cycler (37°C for 30 min)

[_] 2.6. Quick spin, place on ice

3. DNase I Digestion

[_] 3.1. Prepare Master Mix and mix

[_] 3.1.1. DNase I Reaction Buffer 5 µl

[_] 3.1.2. DNase I 2.5 µl

[_] 3.1.3. Nuclease-free water 22.5 µl

[_] 3.2. Add 30 µl of master mix to sample

[_] 3.3. Mix 10 times

[_] 3.4. Quick Spin

[_] 3.5. Put in Thermal cycler (37°C for 30 mn)

[_] 3.6. Quick spin, place on ice

4. RNA Purification Using Agencourt RNAClean XP Beads or NEBNext RNA Sample Purification Beads

[_] 4.1. Add 110 µl of beads and mix 10 times

[_] 4.2. Incubate on ice 15 min

[_] 4.3. Place on Magnet 5 min

[_] 4.4. Remove Supernatant

[_] 4.5. Add 200 µl 80% ethanol, remove after 30 seconds

[_] 4.6. Repeat Step 4.5 once

[_] 4.7. Air dry for up to 5 min

[_] 4.8. Add 8 µl of nuclease-free water and mix 10 times; wait 2 min

[_] 4.9. Place on magnet 5 min

[_] 4.10. Transfer 6 µl to new tube

[_] 4.11. Place on ice or store

Revision History

REVISION #	DESCRIPTION	DATE
2.0	Component change: The name, part # and formulation of RNase H has changed.	11/15
3.0	Protocol edits, renumbering and Quick Checklist	8/16
4.0	E6350 Kit was created and merged with E6310.	4/17
5.0	Required materials not included has additional materials added. Workflow diagram was updated. RNA Sample Recommendations was updated and new text added. Protocol was updated. FAQs were deleted but kept on web page. Kit components were placed in table styles for E6310 as well as E6350.	12/18
6.0	Updated to new manual format.	2/20

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