NEBNext® rRNA Depletion Kit v2 (Human/Mouse/Rat)

NEB #E7400 S/L/X, #E7405 S/L/X

6/24/96 reactions

Version 3.0_8/22

Table of Contents

The Kit Includes

The volumes provided are sufficient for preparation of up to 6 reactions (NEB #E7400S/#E7405S) 24 reactions (NEB #E7400L/#E7405L) and 96 reactions (NEB #E7400X/#E7405X).

Package 1: Store at –20°C.

- (white) NEBNext v2 rRNA Depletion Solution
- (white) NEBNext Probe Hybridization Buffer
- (white) NEBNext Thermostable RNase H
- (white) RNase H Reaction Buffer
- (white) NEBNext DNase I
- (white) DNase I Reaction Buffer

Nuclease-free Water

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

Supplied only with NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) with RNA Sample Purification Beads, NEB #E7405.

NEBNext RNA Sample Purification Beads

Required Materials Not Included

- Pipettes
- Magnetic rack (NEB #S1515S), magnetic plate (Alpaqua® cat. #A001322) or equivalent
- 80% Ethanol (freshly prepared)
- Thin wall 200 µl PCR tubes (For example Tempassure PCR flex-free 8-tube strips USA Scientific #1402-4708)
- Microcentrifuge
- Vortex mixer
- Thermal cycler
- Bioanalyzer®, TapeStation® (Agilent Technologies, Inc.) or similar instrument and consumables

For NEB #E7400 only:

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

For NEB #E7760 & NEB #E7770:

- SPRISelect Reagent Kit (Beckman Coulter, Inc. #B23317) or AMPure® XP Beads (Beckman Coulter, Inc. #A63881)

For use with NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB #E7760/#E7765) & NEBNext Ultra II RNA Library Prep Kit for Illumina (NEB #E7770/#E7775):

- NEBNext Adaptors and Primers:
  - www.neb.com/oligos
  - Alternatively, customer supplied adaptor and primers
    www.neb.com/faq-nonNEB-adaptors

For use with NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB #E7760/#E7765) or the NEBNext Ultra II RNA Library Prep Kit for Illumina (NEB #E7770/#E7775) please use the appropriate protocol the manuals for (NEB #E7760/#E7765) or (NEB #E7770/#E7775). Manuals can be found on NEB.com. Go to the product page, "Protocols, Manuals and Usage Tab", under "Manuals".

Overview

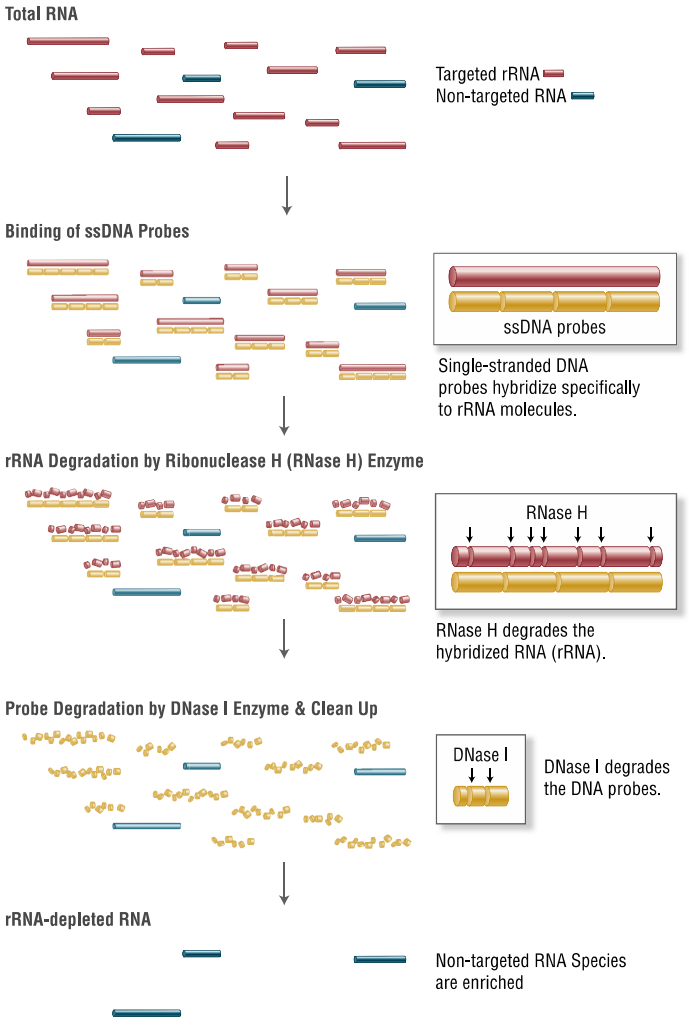
Ribosomal RNA (rRNA) is highly abundant in human, mouse and rat total RNA, and its removal is desirable in order to reveal the biological significance of less abundant transcripts.

The NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) (NEB #E7400, #E7405) employs the NEBNext RNase H-based RNA Depletion Workflow (Figure 1) to enrich for non-ribosomal RNA by depleting the following:

- Cytoplasmic rRNA (5S, 5.8S, 18S, 28S, human ITS, ETS)
- Mitochondrial rRNA (12S and 16S)

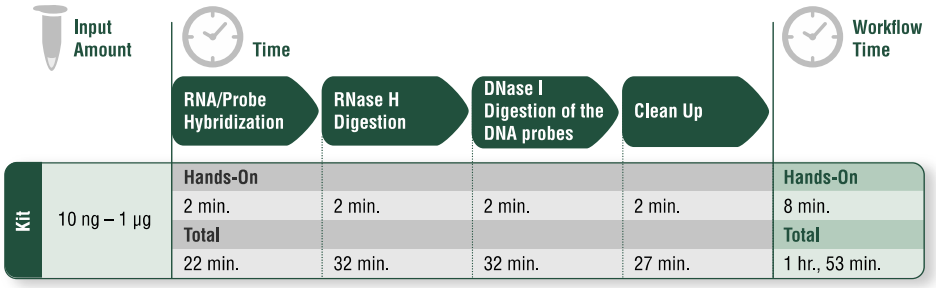
The kit is effective with human, mouse, and rat total RNA preparations, both intact and degraded.

Figure 1. NEBNext RNase H-based rRNA Depletion Workflow.



DNA probes designed against unwanted RNAs (e.g., rRNA) are hybridized to total RNA, followed by RNase H digestion where the enzyme recognizes the RNA:DNA hybrid and degrades the targeted RNA. Finally, the DNA probes are digested with DNase I and the reaction is cleaned using magnetic beads.

The protocol supports rRNA depletion from 10 ng–1 µg total RNA (intact or degraded) and can be completed in approximately two hours.



Applications

The resulting rRNA-depleted RNA is suitable for RNA-Seq, random-primed cDNA synthesis, or other downstream RNA analysis applications.

NEBNext RNA-Seq Product and Protocol Selection Guide

Following depletion the rRNA depleted material can be used in RNA-Seq applications. The library preparation protocol should be chosen based on the goals of the project and quality of the RNA sample. The NEBNext Ultra II Directional RNA Library Prep Kit (NEB #E7760, #E7765) for Illumina uses the dUTP method to retain strand specificity and has a streamlined, automatable workflow. The NEBNext Ultra II RNA Library Prep (NEB #E7770, #E7775) has a non-directional, streamlined and automatable workflow.

When using the NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) (NEB #E7400, #E7405) for RNA-Seq library preparation with the NEBNext kits listed below please follow the appropriate section in the manuals:

- NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB #E7760, #E7765), Section 2 (Intact or Partially Degraded RNA) and Section 3 [Degraded RNA (e.g., FFPE)] in the NEB #E7760, #E7765 manual.
- NEBNext Ultra II RNA Library Prep Kit for Illumina (NEB #E7770, #E7775), Section 2 (Intact or Partially Degraded RNA) and Section 3 [Degraded RNA (e.g., FFPE)] in the NEB #E7770, #E7775 manual.

Every section in this manual contains a different protocol based on the starting material and application. Please read the RNA sample recommendations and input amount requirements in its entirety before starting the protocol.

Each kit component must pass rigorous quality control standards. For each new lot an entire set of reagents is functionally validated together by construction and sequencing of an indexed transcriptome library on the Illumina sequencing platform.

For larger volume requirements, customized and bulk packaging is available by purchasing through the OEM/Bulks department at NEB. Please contact OEM@neb.com for further information.

Protocol for rRNA Depletion using the NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) (NEB #E7400, #E7405)

Symbols

This caution sign signifies a step in the protocol that has two paths leading to the same end point but is dependent on a user variable, like the type of RNA input.

This is a point where you can safely stop the protocol and store the samples prior to proceeding to the next step in the protocol.

●

Colored bullets indicate the cap color of the reagent to be added.

Keep all of the buffers on ice, unless otherwise indicated.

RNA Sample Requirements

RNA Integrity

Assess the quality of the input RNA by running the RNA sample on an Agilent Bioanalyzer RNA 6000 Nano/Pico Chip to determine the RNA Integrity Number (RIN). Both intact and degraded RNA can be used in the depletion protocol. However, processing of samples for different downstream applications may be impacted by the RIN scores.

RNA Purity

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 or silica column methods such as the Monarch® RNA Cleanup Kit (NEB #T2030). Contaminating DNA can cause inaccurate RNA quantification and impede proper globin mRNA and rRNA removal. Prior to depletion the RNA must be in nuclease free water. Some products, e.g., TURBO DNA-free™ Kit, TURBO™ DNase Treatment and Removal Reagents, do not produce RNA in nuclease free water and are not compatible with NEBNext rRNA depletion. Contaminating DNA can cause inaccurate RNA quantification and impede proper globin mRNA and rRNA removal.

Input Amount

10 ng–1 µg total RNA (DNA free) in a maximum of 11 µl of nuclease-free water, quantified by an RNA-specific dye-assisted fluorometric method (e.g., Qubit®, RiboGreen®), and quality checked by Bioanalyzer.

1. Probe Hybridization to RNA

1.1. Dilute 10 ng–1 µg of total RNA with Nuclease-free Water to a final volume of 11 µl in a PCR tube. Keep the RNA on ice.

1.2. Assemble the following RNA/Probe hybridization reaction **on ice**:

RNA/PROBE HYBRIDIZATION REACTION	VOLUME
Total RNA in Nuclease-free Water (10 ng–1 µg)	11 µl
○ (white) NEBNext v2 rRNA Depletion Solution	2 µl
○ (white) NEBNext Probe Hybridization Buffer	2 µl
Total Volume	15 µl

1.3. Mix thoroughly by gently pipetting up and down at least 10 times. **Note: It is crucial to mix well at this step.**

1.4. Briefly spin down the tube in a microcentrifuge to collect the liquid from the side of the tube.

1.5. Place tube in a pre-heated thermal cycler and run the following program with the heated lid set to 105°C. This program will take approximately 15-20 minutes to complete.

TEMPERATURE	TIME
95°C	2 minutes
Ramp down to 22°C	0.1°C/sec
Hold at 22°C	5 minutes

1.6. Briefly spin down the tube in a microcentrifuge and place on ice. Proceed immediately to RNase H Digestion.

2. RNase H Digestion

2.1. Assemble the following RNase H digestion reaction **on ice**:

RNASE H DIGESTION REACTION	VOLUME
Hybridized RNA (Step 1.6)	15 µl
○ (white) NEBNext RNase H Reaction Buffer	2 µl
○ (white) NEBNext Thermostable RNase H	2 µl
Nuclease-free Water	1 µl
Total Volume	20 µl

2.2. Mix thoroughly by pipetting up and down at least 10 times.

2.3. Briefly spin down the tube in a microcentrifuge.

2.4. Incubate the tube in a pre-heated thermal cycler for **30 minutes at 50°C** with the lid set to 55°C.

2.5. Briefly spin down the tube in a microcentrifuge and place on ice. Proceed immediately to DNase I Digestion.

3. DNase I Digestion

3.1. Assemble the following DNase I digestion reaction **on ice**:

DNASE I DIGESTION REACTION	VOLUME
RNase H treated RNA (Step 2.5)	20 µl
○ (white) DNase I Reaction Buffer	5 µl
○ (white) NEBNext DNase I	2.5 µl
Nuclease-free Water	22.5 µl
Total Volume	50 µl

3.2. Mix thoroughly by pipetting up and down at least 10 times.

3.3. Briefly spin down the tube in a microcentrifuge.

3.4. Incubate in a pre-heated thermal cycler for **30 minutes at 37°C** with the heated lid set to 40°C (or off).

3.5. Briefly spin down the tube in a microcentrifuge and place on ice. Proceed immediately to RNA Purification.

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

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

4.2. Add **90 µl (1.8X)** beads to the RNA sample from Step 3.5 and mix thoroughly by pipetting up and down at least 10 times.

4.3. Incubate for **15 minutes on ice** to bind RNA to the beads.

4.4. Place the tube on a magnetic rack to separate the beads from the supernatant.

4.5. After the solution is clear, carefully remove and discard the supernatant. Be careful not to disturb the beads which contain the RNA.

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

4.7. Repeat Step 4.6 once for a total of two washes.

4.8. Completely remove residual ethanol and air dry the beads for up to 5 minutes while the tube is on the magnetic rack 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 from the magnetic rack. Elute the RNA from the beads by adding **7 µl of Nuclease-free Water**. Mix thoroughly by pipetting up and down at least 10 times and briefly spin the tube.

4.10. Incubate for 2 minutes at room temperature.

4.11. Place the tube on the magnetic rack until the solution is clear (~ 2 minutes).

4.12. Remove 5 µl of the supernatant containing RNA and transfer to a nuclease-free tube.

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

Kit Components

NEB #E7400S Table of Components

NEB #	PRODUCT	VOLUME
E7752-2	NEBNext Thermostable RNase H	0.012 ml
E6312-2	RNase H Reaction Buffer	0.012 ml
E7401-2	NEBNext v2 rRNA Depletion Solution	0.012 ml
E6314-2	NEBNext Probe Hybridization Buffer	0.012 ml
E7753-2	NEBNext DNase I	0.015 ml
E6315-2	DNase I Reaction Buffer	0.03 ml
E6317-2	Nuclease-free Water	0.4 ml

NEB #E7400L Table of Components

NEB #	PRODUCT	VOLUME
E7752-3	NEBNext Thermostable RNase H	0.048 ml
E6312-3	RNase H Reaction Buffer	0.048 ml
E7401-3	NEBNext v2 rRNA Depletion Solution	0.048 ml
E6314-3	NEBNext Probe Hybridization Buffer	0.048 ml
E7753-3	NEBNext DNase I	0.06 ml
E6315-3	DNase I Reaction Buffer	0.120 ml
E6317-3	Nuclease-free Water	1.5 ml

NEB #E7400X Table of Components

NEB #	PRODUCT	VOLUME
E7752-4	NEBNext Thermostable RNase H	0.192 ml
E6312-4	RNase H Reaction Buffer	0.192 ml
E7401-4	NEBNext v2 rRNA Depletion Solution	0.192 ml
E6314-4	NEBNext Probe Hybridization Buffer	0.192 ml
E7753-4	NEBNext DNase I	0.24 ml
E6315-4	DNase I Reaction Buffer	0.48 ml
E6317-4	Nuclease-free Water	6.0 ml

NEB #E7405S Table of Components

NEB #	PRODUCT	VOLUME
E7752-2	NEBNext Thermostable RNase H	0.012 ml
E6312-2	RNase H Reaction Buffer	0.012 ml
E7751-2	NEBNext v2 rRNA Depletion Solution	0.012 ml
E6314-2	NEBNext Probe Hybridization Buffer	0.012 ml
E7753-2	NEBNext DNase I	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 #E7405L Table of Components

NEB #	PRODUCT	VOLUME
E7752-3	NEBNext Thermostable RNase H	0.048 ml
E6312-3	RNase H Reaction Buffer	0.048 ml
E7401-3	NEBNext v2 rRNA Depletion Solution	0.048 ml
E6314-3	NEBNext Probe Hybridization Buffer	0.048 ml
E7753-3	NEBNext DNase I	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 #E7405X Table of Components

NEB #	PRODUCT	VOLUME
E7752-4	NEBNext Thermostable RNase H	0.192 ml
E6312-4	RNase H Reaction Buffer	0.192 ml
E7401-4	NEBNext v2 rRNA Depletion Solution	0.192 ml
E6314-4	NEBNext Probe Hybridization Buffer	0.192 ml
E7753-4	NEBNext DNase I	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 for NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) (NEB #E7400, NEB #E7405)

1. Hybridize the Probes to the RNA

1.1. Assemble Probe/RNA Hybridization Reaction

[_] 1.1.1. Total RNA in nuclease-free water 11 µl

[_] 1.1.2. NEBNext v2 rRNA Depletion Solution 2 µl

[_] 1.1.3. NEBNext Probe Hybridization Buffer 2 µl

[_] 1.2. Mix 10 times

[_] 1.3. Quick spin

[_] 1.4. Run in pre-heated thermal cycler (95°C for 2 min, 95-22°C 0.1°C/sec, 22°C 5 min; heated lid 105°C)

[_] 1.5. Quick spin, place on ice

2. RNase H Digestion

2.1. Assemble RNaseH digestion reaction

[_] 2.1.1. Hybridized RNA 15 µl

[_] 2.1.2. NEBNext Thermostable RNase H 2 µl

[_] 2.1.3. RNase H Reaction Buffer 2 µl

[_] 2.1.4. Nuclease-free water 1 µl

[_] 2.2. Mix 10 times

[_] 2.3. Quick spin

[_] 2.4. Incubate in pre-heated thermal cycler (50°C for 30 min)

[_] 2.5. Quick spin, place on ice

3. DNase I Digestion

3.1. Assemble DNase I digestion reaction

[_] 3.1.1. RNase H treated RNA 20 µl

[_] 3.1.2. DNase I Reaction Buffer 5 µl

[_] 3.1.3. NEBNext DNase I 2.5 µl

[_] 3.1.4. Nuclease-free water 22.5 µl

[_] 3.2. Mix 10 times

[_] 3.3. Quick spin

[_] 3.4. Incubate in pre-heated thermal cycler (37°C for 30 min)

[_] 3.5. Quick spin, place on ice

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

[_] 4.1. Add 90 µl of beads and mix 10 times

[_] 4.2. Incubate on ice 15 min

[_] 4.3. Place on magnetic rack until solution is clear

[_] 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 7 µl of Nuclease-free water and mix 10 times; wait 2 min

[_] 4.9. Place on magnet 5 min

[_] 4.10. Transfer 5 µl to new tube

[_] 4.11. Place on ice or store

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	4/20
2.0	Update protocol	11/20
3.0	Update protocol	8/22

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

This product is covered by one or more patents, trademarks and/or copyrights owned or controlled by New England Biolabs, Inc. The use of trademark symbols does not necessarily indicate that the name is trademarked in the country where it is being read; rather, it indicates where the document was originally developed. For more information about commercial rights, please email us at busdev@neb.com. While NEB develops and validates its products for various applications, the use of this product may require you to obtain additional third party intellectual property rights for certain applications.

AGENCOURT®, AMPURE® and RNACLEAN® are registered trademarks of Beckman Coulter, Inc.
B CORPORATION® is a registered trademark of B Lab IP, LLC, Inc.
BIOANALYZER® and TAPESTATION® are registered trademarks of Agilent Technologies, Inc.
ILLUMINA® is a registered trademark of Illumina, Inc.
ALPAQUA® is a registered trademark of Alpaqua Engineering. LLC
QUBIT® and RIBOGREEN® are registered trademarks of Molecular Probes, Inc.

New England Biolabs, Inc., 240 County Road, Ipswich, MA 01938-2723 Telephone: (978) 927-5054 Toll Free: (USA Orders) 1-800-632-5227 (USA Tech) 1-800-632-7799 Fax: (978) 921-1350 e-mail: info@neb.com