NEBNext® Ultra™ II Non-Directional RNA Second Strand Synthesis Module

NEB #E6111S/L

20/100 reactions

Version 6.0_5/22

Table of Contents

The NEBNext Ultra II Non-Directional RNA Second Strand Synthesis Module Includes

The volumes provided are sufficient for preparation of up to 20 reactions (NEB #E6111S) and 100 reactions (NEB #E6111L). All reagents should be stored at –20°C. Colored bullets represent the color of the cap of the tube containing the reagent.

- (orange) NEBNext Second Strand Synthesis Enzyme Mix
- (orange) NEBNext Second Strand Synthesis Reaction Buffer

The NEBNext Ultra II Non-Directional RNA Second Strand Synthesis Module is Designed for use with the Following:

NEBNext Ultra II RNA First Strand Synthesis Module (NEB #E7771) or
NEBNext RNA First Strand Synthesis Module (NEB #E7525)

NEBNext Multiplex Oligos for Illumina® (NEB.com/oligos)

NEBNext Ultra II End Repair/dA-Tailing Module (NEB #E7546) or
NEBNext Ultra End Repair/dA-Tailing Module (NEB #E7442)

NEBNext Ultra II Ligation Module (NEB #E7595) or
NEBNext Ultra Ligation Module (NEB #E7445)

NEBNext Ultra II Q5 Master Mix (NEB #M0544) or
NEBNext High Fidelity 2X PCR Master Mix (NEB #M0541)

Required Materials Not Included

- 80% Ethanol (freshly prepared)
- Nuclease-free Water
- Magnetic rack/stand (NEB #S1515S, Alpaqua®, cat. #A001322 or equivalent)
- Thermal cycler
- Vortex Mixer
- Microcentrifuge
- SPRIselect® Reagent (Beckman Coulter®, Inc. #B23317) or AMPure® XP Beads (Beckman Coulter, Inc. #A63881)
- DNase RNase free PCR strip tubes (USA Scientific® 1402-1708)

Overview

The NEBNext Ultra II Non-Directional RNA Second Strand Synthesis Module has been optimized to generate double stranded cDNA from first strand cDNA using the NEBNext Ultra II RNA First Strand Synthesis Module (NEB #E7771), or NEBNext RNA First Strand Synthesis Module (NEB #E7525). The dsDNA generated by the NEBNext Ultra II Non-Directional RNA Second Strand Synthesis Module can be subsequently converted to blunt ended DNA fragments using the NEBNext Ultra II End Repair/dA-Tailing Module (NEB #E7546).

Each module component must pass rigorous quality control standards, and for each new lot the entire set of reagents is functionally validated together with NEB #E7111, #E7546, #E7595 and #M0544 to construct an indexed transcriptome library that is sequenced on an 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

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.

Note: This protocol has changed to be compatible with the NEBNext Ultra II RNA Workflow. If you need access to the previous version of the manual, please contact info@neb.com.

Starting Material: 20 µl of first strand cDNA synthesized with the NEBNext Ultra II RNA First Strand RNA Synthesis Module (#E7771, Chapter 2).

1. Second Strand cDNA Synthesis

1.1. Assemble the second strand cDNA synthesis reaction **on ice** by adding the following components into the first strand synthesis reaction product.

COMPONENT	VOLUME
First-strand Synthesis Product	20 µl
● (orange) NEBNext Second Strand Synthesis Reaction Buffer	8 µl
● (orange) NEBNext Second Strand Synthesis Enzyme Mix	4 µl
Nuclease-free Water	48 µl
Total Volume	80 µl

1.2. Keeping the tube on ice, mix thoroughly by pipetting the reaction up and down at least 10 times.

1.3. Incubate in a thermocycler for **1 hour at 16°C** with the heated lid set at ≤ 40°C (or off).

2. Purification of Double-stranded cDNA using SPRIselect Beads or NEBNext Sample Purification Beads

2.1. Vortex SPRIselect Beads or NEBNext Sample Purification Beads to resuspend.

2.2. Add 144 µl (1.8X) of resuspended beads to the second strand synthesis reaction (~80 µl). Mix well on a vortex mixer or by pipetting up and down at least 10 times.

2.3. Incubate for 5 minutes at room temperature.

2.4. Briefly spin the tube in a microcentrifuge to collect any sample from the sides of the tube. Place the tube on a magnetic rack to separate beads from the supernatant. After the solution is clear, carefully remove and discard the supernatant. Be careful not to disturb the beads, which contain DNA. **(Caution: Do not discard beads)**

2.5. Add 200 µl of freshly prepared 80% ethanol to the tube while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.

2.6. Repeat Step 2.5 once for a total of 2 washing steps.

2.7. Air dry the beads for up to 5 minutes while the tube is on the magnetic rack with the lid open.

Caution: Do not overdry the beads. This may result in lower recovery of DNA 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.

2.8. Remove the tube from the magnet. Elute the DNA target from the beads by adding 53 µl 0.1X TE Buffer to the beads. Mix well on a vortex mixer or by pipetting up and down ten times. Briefly spin the tube and incubate for 2 minutes at room temperature. Place the tube on the magnetic rack until the solution is clear.

2.9 Remove 50 µl of the supernatant and transfer to a clean nuclease-free PCR tube.

Note: If you need to stop at this point in the protocol, samples can be stored at –20°C.

2.10 Proceed to the NEBNext Ultra II End Repair/dA-Tailing Module (NEB #E7546).

Kit Components

Each set of reagents is functionally validated together with NEB #E7111, NEB #E7546, NEB #E7595, and NEB #M0544, and compared to the previous lot through construction of libraries using the minimum and maximum amount of Universal Human Reference Total RNA. The previous and current lots are sequenced together on the same Illumina flow cell and compared across various sequence metrics including individual transcript abundances, 5´→3´ transcript coverage, and fraction of reads mapping to the reference

NEB #E6111S Table of Components

NEB #	PRODUCT	VOLUME
E6112A	NEBNext Second Strand Synthesis Enzyme Mix	0.080 ml
E6113A	NEBNext Second Strand Synthesis Reaction Buffer	0.160 ml

NEB #E6111L Table of Components

NEB #	PRODUCT	VOLUME
E6112AA	NEBNext Second Strand Synthesis Enzyme Mix	0.400 ml
E6113AA	NEBNext Second Strand Synthesis Reaction Buffer	0.800 ml

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	
2.0	Name of this kit is changed to NEBNext Ultra II Non-directional RNA Second Strand Synthesis Module. Protocol changed to be compatible with the NEBNext Ultra II RNA Workflow.	1/17
3.0	Add "NEBNext Ultra II Directional RNA Second Strand Synthesis Module is designed for use with other kits listed." Add edits to protocol.	4/18
4.0	New format applied.	9/19
5.0	Update Step 2.8, add 2.9 and 2.10	4/20
6.0	Added Required materials not included	5/22

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