NEBNext® Ultra™ II Ligation Module

NEB #E7595S/L

24/96 reactions

Version 4.0_9/22

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The NEBNext Ultra II Ligation Module Includes

The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E7595S) and 96 reactions (NEB #E7595L). All reagents should be stored at –20°C. Colored bullets represent the color of the cap of the tube containing the reagent.

- (red) NEBNext Ultra II Ligation Master Mix
- (red) NEBNext Ligation Enhancer

The NEBNext Ultra II Ligation Module is Designed for use with the Following

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

NEBNext Ultra II FS DNA Module (NEB #E7810)

NEBNext Ultra II Q5® Master Mix (NEB #M0544)

NEBNext Oligo kit options can be found at neb.com/oligos

Alternatively, customer supplied adaptor and primers can be used, please see information in link below:
https://www.neb.com/faqs/2019/03/08/can-i-use-this-nebnext-kit-with-adaptors-and-primers-from-other-vendors-than-neb

Please note: This manual is not for use with UNIQUE DUAL INDEX UMI ADAPTORS.

Required Materials Not Included

- Thermal cycler
- AMPure® XP Beads (Beckman Coulter, Inc. #A63881) or SPRIselect® Reagent Kit (Beckman Coulter, Inc. #B23317)
- 10 mM Tris-HCl, pH 7.5–8.0 or 0.1 µM Tris-HCl, pH 8.0 (for adaptor dilution) or NEB #B1430
- Vortex Mixer
- Microcentrifuge
- DNase RNase free PCR strip tubes (USA Scientific® 14021708)
- Magnetic rack/stand (NEB #S1515, Alpaqua®, cat. #A001322 or equivalent)

Applications

The NEBNext Ultra II Ligation Module is optimized for use with the NEBNext Ultra II End Repair/dA-Tailing Module (NEB #E7546)/ or the NEBNext Ultra II FS DNA Module (NEB #E7810).

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 #E7546 or NEB #E7810 along with NEB #M0544 to construct indexed libraries that are 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.

Section 1
Protocol for use with NEBNext Ultra II End Repair/dA-Tailing Module (NEB #E7546)

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 amount of input DNA.

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.

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

Starting Material
500 pg–1 µg fragmented DNA that has been end repaired and dA-Tailed using the NEBNext End Repair/dA-Tailing Module (NEB #E7546).

If DNA input is ≤ 100 ng, dilute the NEBNext Adaptor for Illumina in 10 mM Tris-HCl, pH 7.5-8.0 with 10 mM NaCl as indicated in Table 1.1.

Note: The appropriate adaptor dilution for your sample input and type may need to be optimized experimentally. The dilutions provided here are a general starting point.

Table 1.1: Adaptor Dilution.

INPUT	ADAPTOR DILUTION (VOLUME OF ADAPTOR: TOTAL VOLUME)	WORKING ADAPTOR CONCENTRATION
1 µg–101 ng	No Dilution	15 µM
100 ng–5 ng	10-Fold (1:10)	1.5 µM
less than 5 ng	25-Fold (1:25)	0.6 µM

1.1. Add the following components directly to the End Prep Reaction Mixture:

COMPONENT	VOLUME (µl) PER REACTION
End Prep Reaction Mixture	60 µl
• (red) NEBNext Adaptor for Illumina**	2.5 µl
• (red) NEBNext Ultra II Ligation Master Mix*	30 µl
• (red) NEBNext Ligation Enhancer	1 µl
Total Volume	93.5 µl

*Mix the Ultra II Ligation Master Mix by pipetting up and down several times prior to adding to the reaction.

**The NEBNext adaptor is provided in the NEBNext Oligo kit options, which can be found at neb.com/oligos.

Note: The Ligation Master Mix and Ligation Enhancer can be mixed ahead of time and is stable for at least 8 hours @ 4°C. Do not premix the Ligation Master Mix, Ligation Enhancer and adaptor prior to use in the Adaptor Ligation Step.

1.2. Set a 100 µl or 200 µl pipette to 80 µl and then pipette the entire volume up and down at least 10 times to mix thoroughly. Perform a quick spin to collect all liquid from the sides of the tube.
(Caution: The NEBNext Ultra II Ligation Master Mix is very viscous. Care should be taken to ensure adequate mixing of the ligation reaction, as incomplete mixing will result in reduced ligation efficiency. The presence of a small amount of bubbles will not interfere with performance).

1.3. Incubate at 20°C for 15 minutes in a thermal cycler with the heated lid off.

1.4. Add 3 µl of • (red) USER® Enzyme to the ligation mixture from Step 1.3.

Note: Steps 1.4 and 1.5 are only required for use with non-indexed NEBNext Adaptors. USER enzyme can be found in most NEBNext oligo kits, all options can be found on the neb.com/oligos page. If you are using the indexed UMI adaptor, USER is not needed. Please see corresponding manual for use with UMI on the E7395 product page under the protocols, manuals, and usage tab.

1.5. Mix well and incubate at 37°C for 15 minutes with the heated lid set to ≥ 47°C.

1.6. DNA is now ready for size selection or cleanup.

Note: Please see NEB #E7645/#E7103 manual for recommended size selection/cleanup and PCR amplification protocols.

Samples can be stored overnight at –20°C.

Section 2
Protocol for use with NEBNext Ultra II FS DNA Module (NEB #E7810)

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 amount of input DNA.

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.

Starting Material
100 pg–500 ng fragmented, end repaired and dA-Tailed DNA generated using the NEBNext Ultra II FS DNA Module (NEB #E7810).

If DNA input is ≤ 100 ng, dilute the • (red) NEBNext Adaptor for Illumina in 10 mM Tris-HCl, pH 7.5-8.0 with 10 mM NaCl as indicated in Table 2.1.

Note: The appropriate adaptor dilution for your sample input and type may need to be optimized experimentally. The dilutions provided here are a general starting point.

Table 2.1 Adaptor Dilution.

INPUT	ADAPTOR DILUTION (VOLUME OF ADAPTOR: TOTAL VOLUME)	WORKING ADAPTOR CONCENTRATION
100 ng–500 ng	No Dilution	15 µM
5 ng–99 ng	10-Fold (1:10)	1.5 µM
less than 5 ng	25-Fold (1:25)	0.6 µM

2.1. Add the following components directly to the FS Reaction Mixture

COMPONENT	VOLUME (µl) PER REACTION
FS Reaction Mixture	35 µl
• (red) NEBNext Adaptor for Illumina**	2.5 µl
• (red) NEBNext Ultra II Ligation Master Mix*	30 µl
• (red) NEBNext Ligation Enhancer	1 µl
Total Volume	68.5 µl

*Mix the Ultra II Ligation Master Mix by pipetting up and down several times prior to adding to the reaction.
**The NEBNext adaptor is provided in the NEBNext Oligo kit options, which can be found at neb.com/oligos.

Note: The Ligation Master Mix and Ligation Enhancer can be mixed ahead of time and is stable for at least 8 hours @ 4°C. We do not recommend premixing the Ligation Master Mix, Ligation Enhancer and adaptor prior to use in the Adaptor Ligation Step.

2.2. Set a 100 µl or 200 µl pipette to 50 µl and then pipette the entire volume up and down at least 10 times to mix thoroughly. Perform a quick spin to collect all liquid from the sides of the tube.
(Caution: The NEBNext Ultra II Ligation Master Mix is very viscous. Care should be taken to ensure adequate mixing of the ligation reaction, as incomplete mixing will result in reduced ligation efficiency. The presence of a small amount of bubbles will not interfere with performance).

2.3. Incubate at 20°C for 15 minutes in a thermocycler with the heated lid off.

2.4. Add 3 µl of • (red) USER® Enzyme to the ligation mixture from Step 2.3.

Note: Steps 2.4 and 2.5 are only required for use with NEBNext Adaptors. USER enzyme can be found in most NEBNext oligo kits, all options can be found on the neb.com/oligos page. If you are using the indexed UMI adaptor, USER is not needed. Please see corresponding manual for use with UMI on the E7395 product page under the protocols, manuals, and usage tab**.**

2.5. Mix well and incubate at 37°C for 15 minutes with the heated lid set to ≥ 47°C.

2.6. DNA is now ready for size selection or cleanup.

Note: Please see NEB #E7805/#E6177 manual for recommended size selection/cleanup and PCR amplification protocols.

Samples can be stored overnight at –20°C.

Kit Components

NEB #E7595S Table of Components

NEB #	PRODUCT	VOLUME
E7648A	NEBNext Ultra II Ligation Master Mix	0.72 ml
E7374A	NEBNext Ligation Enhancer	0.024 ml

NEB #E7595L Table of Components

NEB #	PRODUCT	VOLUME
E7648AA	NEBNext Ultra II Ligation Master Mix	3 x 0.960 ml
E7374AA	NEBNext Ligation Enhancer	0.096 ml

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	9/15
1.1	Updated "Designed for Use" to include NEB #E7710 and NEB #E7730	6/16
2.0	Updated to include a protocol for use with NEBNext Ultra II FS DNA Module (NEB #E7810). Inserted chapter one and chapter two and applied chapter numbering.	11/17
3.0	New Format Applied.	4/20
4.0	Update protocol and required materials not included	9/22