NEBNext® Ultra™ Ligation Module

NEB #E7445L

96 reactions

Version 4.0_6/22

Table of Contents

The NEBNext Ultra Ligation Module Includes:

The volumes provided are sufficient for preparation of up to 96 reactions (NEB #E7445L). All reagents should be stored at –20°C. Colored bullets represent the color of the cap of the tube containing the reagent.

- (red) Blunt/TA Ligase Master Mix
- (red) NEBNext Ligation Enhancer

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

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

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

NEBNext Q5 Hot Start HiFi PCR Master Mix (NEB #M0543)

NEBNext High-Fidelity 2X PCR Master Mix (NEB #M0541)

Applications

The NEBNext Ultra Ligation Module is designed for use with the NEBNext Multiplex Oligos for Illumina.

Lot Control: The lots provided in the NEBNext Ultra Ligation Module are managed separately and qualified by additional functional validation. Individual reagents undergo standard enzyme activity and quality control assays, and also meet stringent criteria in the additional quality controls listed on each individual component page.

Functionally Validated: Each set of reagents is functionally validated together through construction and sequencing of an indexed DNA 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 DNA

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 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.

Starting Material

5 ng–1 µg of fragmented DNA that has been end repaired and dA-tailed using the NEBNext End Repair/dA-Tailing Module (NEB #E7442).

If DNA input prior to End Repair is < 100 ng, dilute the ● (red) NEBNext Adaptor for Illumina 1:10 in 10 mM Tris-HCl pH 7.5–8.0 or 10 mM Tris-HCl pH 7.5–8.0 with 10 mM NaCl to a final concentration of 1.5 µM. Use immediately.*

1. Add the following components directly to the End Prep reaction mixture (65 µl) and mix well:

COMPONENT	VOLUME (µl) PER REACTION
● (red) NEBNext Adaptor for Illumina*	2.5 µl
● (red) Ligation Enhancer	1 µl
● (red) Blunt/TA Ligase Master Mix**	15 µl
Total Volume	83.5 µl

* The NEBNext adaptor is provided in NEBNext oligos kit. NEB has several oligo kit options, which are supplied separately from the library prep kit.

** Mix the Blunt/TA Ligase Master Mix by pipetting up and down several times prior to adding to the reaction.

Note: The Ligation Enhancer and Blunt/TA Ligase Master Mix can be mixed ahead of time and is stable for at least 8 hours at 4°C. We do not recommend adding adaptor to a premix in the Adaptor Ligation Step. For best results add adaptor last and mix well immediately or premix adaptor and sample and then add the other ligation reagents.

2. Set a 100 µl or 200 µl pipette to 80 µl and then pipette the entire volume up and down to mix thoroughly. Perform a quick spin to collect all liquid from the sides of the tube. **(Caution: The Blunt/TA Ligase Master Mix is 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).**
3. Incubate at 20°C for 15 minutes in a thermal cycler.
4. Add 3 µl of USER® enzyme to the ligation mixture from Step 3.

   Note: This step is only required for use with NEBNext Adaptors. USER Enzyme can be found in the NEBNext Multiplex Oligo kits.
5. Mix well and incubate at 37°C for 15 minutes with the heated lid set to ≥ 47°C.
6. DNA is now ready for size selection or clean-up using Agencourt® AMPure® XP beads.

   Note: Please see NEB #E7370 manual for recommended size-selection/ cleanup and PCR Amplification Protocols.

Protocol for Directional RNA

Perform Adaptor Ligation

Dilute the • (red) NEBNext Adaptor for Illumina* 1:10 in 10 mM Tris-HCl pH 7.5–8.0 or 10 mM Tris-HCl pH 7.5–8.0 with 10 mM NaCl to a final concentration of 1.5 µM. Use immediately.

1. Add the following components directly to the End Prep reaction mixture (65 µl) and mix well:

COMPONENT	VOLUME (µl) PER REACTION
• (red) NEBNext Adaptor (1.5 µM)*	1 µl
Nuclease-free Water	2.5 µl
• (red) Blunt/TA Ligase Master Mix	15 µl
Total Volume	83.5 µl

*The adaptor is provided in NEBNext Multiplex Oligos for Illumina.

2. Mix by pipetting, followed by a quick spin to collect all liquid from the sides of the tube.
3. Incubate at 20°C for 15 minutes in a thermal cycler.

   Note: USER step is performed during the PCR reaction.
4. Proceed to desired size selection method.

   Note: Please see NEB #E7420 manual for recommended size-selection/cleanup, USER cleavage and PCR Amplification protocols.

Kit Components

NEB #E7445L Table of Components

NEB #	PRODUCT	VOLUME
E7374AA	NEBNext Ligation Enhancer	0.096 ml
E7373AA	Blunt/TA Ligase Master Mix	1.44 ml

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	
1.1	Updated "Designed for Use", to include NEB #E7710 and NEB #E7730	6/16
2.0	Create "Kit Component – Table of Components" for small and large size kits. Delete individual component information pages	4/18
3.0	New format applied.	1/20
4.0	Remove small size and update protocol	6/22

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