

LIBRARY PREPARATION

NEBNext[®] Ultra Ligation Module

Instruction Manual

NEB #E7445S/L
24/96 reactions
Version 2.0 4/18



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BioLabs[®] Inc.

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drive DISCOVERY
stay GENUINE

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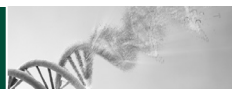


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The NEBNext Ultra Ligation Module Includes:

The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E7445S) and 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 Products:

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

NEBNext Singleplex or Multiplex Oligos for Illumina®
(NEB #E7350, #E7335, #E7500, #E7710, #E7730, #E6609 or #E7600)

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 Singleplex or 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 multiple paths leading to the same end point but is dependent on a user variable, like the amount of input DNA.

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)

Adaptor Ligation



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:

● (red) Blunt/TA Ligase Master Mix	15 µl
● (red) NEBNext Adaptor for Illumina*	⚠ 2.5 µl
● (red) Ligation Enhancer	1 µl
<hr/>	
Total volume	83.5 µl

*The NEBNext adaptor is provided in NEBNext Singleplex (NEB #E7350) or Multiplex (NEB #E7335, #E7500, #E7710, #E7730, #E6609, #E7600) 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 thermocycler.
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 used in the NEBNext Singleplex (NEB #E7350) or Multiplex (NEB #E7335, #E7500, #E7710, #E7730, #E6609 or #E7600) Oligos for Illumina.
5. Mix well and incubate at 37°C for 15 minutes.
6. DNA is now ready for size selection or clean-up using Agencourt® AMPure XP beads or a Qiagen® PCR column.
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:

● (red) Blunt/TA Ligase Master Mix	15 µl
● (red) NEBNext Adaptor (1.5 µM)*	1 µl
Nuclease-free Water	2.5 µl
Total volume	83.5 µl

*The adaptor is provided in NEBNext Singleplex (NEB #E7350) or NEBNext Multiplex (NEB #E7335, #E7500, #E7710, #E7730, #E6609) Oligos for Illumina.

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

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 #E7445S Table of Components

NEB #	PRODUCT	VOLUME
E7374A	NEBNext Ligation Enhancer	0.024 ml
E7373A	Blunt/TA Ligase Master Mix	0.360 ml

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



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