

## NEBNext<sup>®</sup> Ultra<sup>™</sup> II End Repair/dA-Tailing Module

NEB #E7546S/L

24/96 reactions

Version 4.0\_9/22

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### The NEBNext Ultra II End Repair/dA-Tailing Module Includes

The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E7546S) and 96 reactions (NEB #E7546L). All reagents should be stored at  $-20^{\circ}\text{C}$ . Colored bullets represent the color of the cap of the tube containing the reagent.

- (green) NEBNext Ultra II End Prep Reaction Buffer
- (green) NEBNext Ultra II End Prep Enzyme Mix

### The NEBNext Ultra II End Repair/dA-Tailing Module is Designed for use with the Following:

NEBNext Ultra II Ligation Module (NEB #E7595)

NEBNext Ultra II Q5<sup>®</sup> Master Mix (NEB #M0544)NEBNext Oligo kit options can be found at [neb.com/oligos](http://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:** A separate manual exists for UNIQUE DUAL INDEX UMI ADAPTORS.

### Required Materials Not Included

- Thermal cycler
- Vortex Mixer
- Microcentrifuge
- DNase RNase free PCR strip tubes (USA Scientific<sup>®</sup> 1402-1708)

### Applications

The NEBNext Ultra II End Repair/dA-Tailing Module is optimized to convert 500 pg-1  $\mu\text{g}$  of fragmented DNA to repaired DNA having 5' phosphorylated, 3' dA-tailed ends.

**Lot Control:** The lots provided in the NEBNext Ultra II End Repair/dA-Tailing Module for Illumina 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](mailto:OEM@neb.com) for further information.

## Protocol for use with NEBNext Ultra II End Repair/dA-Tailing Module

### 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:** 500 pg–1 µg fragmented DNA. We recommend that DNA be sheared in 1X TE. If the DNA volume post shearing is less than 50 µl, add 1X TE to a final volume of 50 µl. Alternatively, 10 mM Tris-HCl, pH 8.0 or 0.1X TE can be used.

### 1. NEBNext End Prep

1.1. Add the following components to a sterile nuclease-free tube:

| END PREP REACTION                                   | VOLUME |
|---|--------|
| • (green) NEBNext Ultra II End Prep Reaction Buffer | 7 µl   |
| • (green) NEBNext Ultra II End Prep Enzyme Mix      | 3 µl   |
| Fragmented DNA                                      | 50 µl  |
| Total Volume  | 60 µl  |

1.2. 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.

**Note: It is important to mix well. The presence of a small amount of bubbles will not interfere with performance.**

1.3. Place in a thermal cycler, with the heated lid set to  $\geq 75^{\circ}\text{C}$ , and run the following program:

30 minutes @  $20^{\circ}\text{C}$

30 minutes @  $65^{\circ}\text{C}$

Hold at  $4^{\circ}\text{C}$



**If necessary, samples can be stored at  $-20^{\circ}\text{C}$ ; however, a slight loss in yield (~20%) may be observed. We recommend continuing with adaptor ligation before stopping.**

1.4. Proceed directly to NEBNext Ultra II Ligation Module (NEB #E7595)

## Kit Components

### NEB #E7546S Table of Components

| NEB #  | PRODUCT                                   | VOLUME   |
|--------|---|----------|
| E7646A | NEBNext Ultra II End Prep Enzyme Mix      | 0.072 ml |
| E7647A | NEBNext Ultra II End Prep Reaction Buffer | 0.168 ml |

### NEB #E7546L Table of Components

| NEB #   | PRODUCT                                   | VOLUME   |
|---------|---|----------|
| E7646AA | NEBNext Ultra II End Prep Enzyme Mix      | 0.288 ml |
| E7647AA | NEBNext Ultra II End Prep Reaction Buffer | 0.672 ml |

## Revision History

| REVISION # | DESCRIPTION  | DATE |
|------------|--|------|
| 1.0        | N/A  | 8/15 |
| 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.  | 3/20 |
| 4.0        | Update protocol and required materials not included  | 9/22 |

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