

NEBNext[®] Ultra[™] End Repair/dA-Tailing Module

NEB #E7442L

96 reactions Version 4.0_6/22

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The NEBNext Ultra End Repair/dA-Tailing Module Includes:

The volumes provided are sufficient for preparation of up to 96 reactions (NEB #E7442L). All reagents should be stored at -20° C. Colored bullets represent the color of the cap of the tube containing the reagent.

- (green) NEBNext End Repair Reaction Buffer
- (green) NEBNext End Prep Enzyme Mix

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

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

NEBNext Ultra Ligation Module (NEB #E7445)

NEBNext High Fidelity 2X PCR Master Mix (NEB #M0541) or NEBNext Q5 Hot Start HiFi PCR Master Mix (NEB #M0543)

Required Materials Not Included

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

Applications

The NEBNext End Prep module is optimized to convert 5 ng-1 µg of fragmented DNA to repaired DNA having 5´ phosphorylated, dA-tailed ends.

Lot Control: The lots provided in the NEBNext Ultra End Prep Module are managed separately and qualified by additional functional validation. Individual reagents undergo standard enzyme activity and quality control assays, and 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

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 DNA 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.

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

Starting Material: 5 ng-1 µg fragmented DNA

NEBNext End Prep

1. Mix the following components in a sterile, nuclease-free tube:

COMPONENT	VOLUME
• (green) NEBNext End Prep Enzyme Mix	3.0 µl
• (green) NEBNext End Repair Reaction Buffer	6.5 µl
Fragmented DNA	55.5 μl
Total Volume	65 µl

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

30 minutes @ 20°C

30 minutes @ 65°C

Hold at 4°C

4. Proceed directly to NEBNext Ultra Ligation Module (NEB #E7445).

Kit Components

NEB #E7442L Table of Components

NEB#	PRODUCT	VOLUME
E7371AA	NEBNext End Prep Enzyme Mix	0.288 ml
E7372AA	NEBNext End Repair Reaction Buffer	0.624 ml

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	2/13
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

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