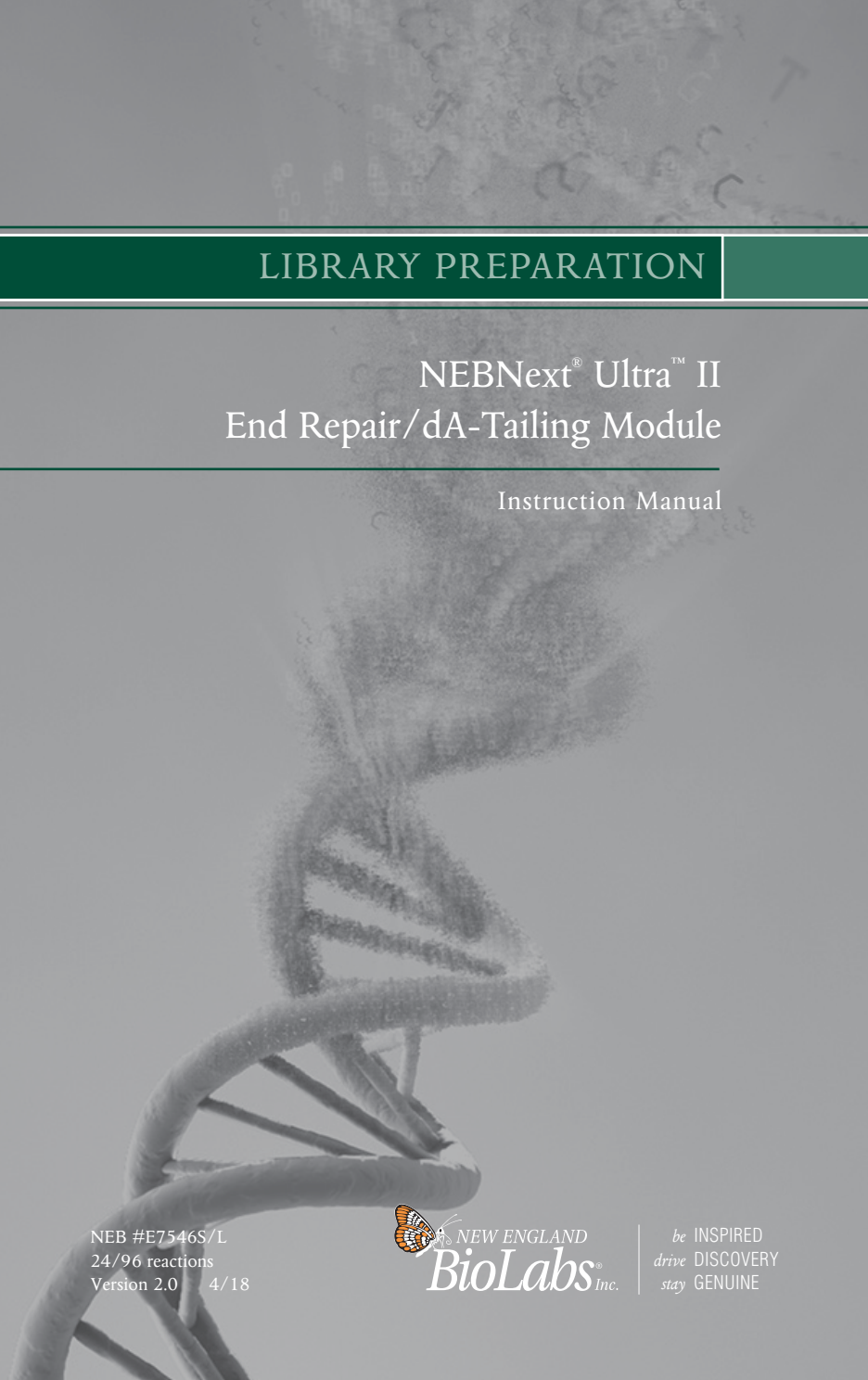
LIBRARY PREPARATION

NEBNext® Ultra™ II End Repair/dA-Tailing Module

Instruction Manual

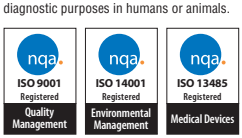


NEB #E7546S/L
24/96 reactions
Version 2.0 | 4/18

NEW ENGLAND BioLabs® Inc.

be INSPIRED
drive DISCOVERY
stay GENUINE

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.



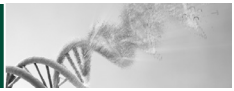


Table of Contents:

The NEBNext Ultra 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°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 Singleplex or Multiplex Oligos for Illumina®
(NEB #E7350, #E7335, #E7500, #E7600 or #E7535)

NEBNext Ultra II Ligation Module (NEB #E7595)

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

Applications:

The NEBNext Ultra II End Repair/dA-Tailing Module is optimized to convert 500 pg-1 µg 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 for further information.

Protocol:

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.

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

Stopping points in the protocol.

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.

1.1 NEBNext End Prep

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

(green) NEBNext Ultra II End Prep Enzyme Mix	3 µl
(green) NEBNext Ultra II End Prep Reaction Buffer	7 µl
Fragmented DNA	50 µl
Total volume	60 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.

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

3. Place in a thermocycler, with the heated lid set to ≥ 75°C, and run the following program:

   30 minutes @ 20°C

   30 minutes @ 65°C

   Hold at 4°C

If necessary, samples can be stored at –20°C; however, a slight loss in yield (~20%) may be observed. We recommend continuing with adaptor ligation before stopping.

4. Proceed directly to NEBNext Ultra II Ligation Module (NEB #E7595) (available in September 2015).

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

USA
New England Biolabs, Inc.
240 County Road
Ipswich, MA 01938-2723
Telephone: (978) 927-5054
Toll Free: (USA Orders) 1-800-632-5227
Toll Free: (USA Tech) 1-800-632-7799
Fax: (978) 921-1350
e-mail: info@neb.com
www.neb.com

CANADA
New England Biolabs, Ltd.
Telephone: (905) 665-4632
Toll Free: 1-800-387-1095
Fax: (905) 665-4635
Fax Toll Free: 1-800-563-3789
e-mail: info.ca@neb.com
www.neb.ca

CHINA
New England Biolabs (Beijing), Ltd.
Telephone: 010-82378265/82378266
Fax: 010-82378262
e-mail: info@neb-china.com
www.neb-china.com

FRANCE
New England Biolabs France
Free Call: 0800-100-632
Free Fax: 0800-100-610
e-mail: info.fr@neb.com
www.neb-online.fr

GERMANY & AUSTRIA
New England Biolabs GmbH
Telephone: +49/(0)69/305 23140
Free Call: 0800/246 5227 (Germany)
Free Call: 00800/246 52277 (Austria)
Fax: +49/(0)69/305 23149
Free Fax: 0800/246 5229 (Germany)
e-mail: info.de@neb.com
www.neb-online.de

JAPAN
New England Biolabs Japan, Inc.
Telephone: +81 (0)3 5669 6191
Fax: +81 (0)3 5669 6192
e-mail: info.jp@neb.com
www.nebj.jp

SINGAPORE
New England Biolabs Pte. Ltd.
Telephone: +65 638 59623
Fax: +65 638 59617
e-mail: sales.sg@neb.com
www.neb.sg

UNITED KINGDOM
New England Biolabs (UK) Ltd.
Telephone: (01462) 420616
Call Free: 0800 318486
Fax: (01462) 421057
Fax Free: 0800 435682
e-mail: info.uk@neb.com
www.neb.uk.com

NEW ENGLAND BioLabs® Inc.