# NEBNext® End Repair Module

NEB #E6050S/L

20/100 reactions

Version 5.0_6/22

## Table of Contents



## The NEBNext End Repair Module Includes

*The volumes provided are sufficient for preparation of up to 20 reactions (NEB #E6050S) and 100 reactions (NEB #E6050L). All reagents should be stored at –20°C.*

NEBNext End Repair Enzyme Mix

NEBNext End Repair Reaction Buffer

## The NEBNext End Repair Module is Designed for use with the Following:

NEBNext Quick Ligation Module (NEB #E6056)

NEBNext dA-Tailing Module (NEB #E6053)

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

NEBNext Oligo kit options can be found at 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:** This manual is not for use with UNIQUE DUAL INDEX UMI ADAPTORS.

## Required Materials Not Included

- Thermal cycler
- AMPure® XP Beads (Beckman Coulter, Inc. #A63881) or SPRIselect® Reagent Kit (Beckman Coulter, Inc. #B23317)
- Vortex Mixer
- Microcentrifuge
- DNase RNase free PCR strip tubes (USA Scientific® 1402-1708)
- Magnetic rack/stand (NEB #S1515, Alpaqua®, cat. #A001322 or equivalent)
- 10 mM Tris-HCl or 0.1X TE
- Bioanalyzer® (Agilent Technologies, Inc.) or similar instrument and consumables

## Description

The NEBNext End Repair Module has been optimized to convert 1 µg–5 µg of fragmented DNA to blunt-ended DNA having 5´ phosphates, and 3´-hydroxyls. The module is optimized for use with the NEBNext dA-Tailing Module (NEB #E6053), and is part of the original standard DNA library prep workflow for Illumina sequencing.

Each kit component must pass rigorous quality control standards, and for each new lot the entire set of reagents is functionally validated together by construction and sequencing of an indexed 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.

## Applications

**DNA sample preparation**

**End repair of 1-5 µg fragmented DNA**

## Advantages

- Efficient – Converts 1–5 µg fragmented DNA to blunt ended DNA
- Convenient – Reactions are provided in master mix format to reduce steps during DNA sample prep workflows
- Automation Friendly

## Protocol for use with NEBNext End Repair Module

**Starting Material**: 1–5 µg of DNA Fragmented to 100–1,000 bp in ≤ 85 µl

**1. NEBNext End Repair**

1.1. Mix the following components in a sterile microfuge tube:

| COMPONENT | VOLUME (µl) PER REACTION |
|---|---|
| Fragmented DNA | variable |
| NEBNext End Repair Reaction Buffer | 10 µl |
| NEBNext End Repair Enzyme Mix | 5 µl |
| Sterile $H_20$ for a final volume of 100 µl | variable |
| Total Volume | 100 µl |

1.2. Incubate in a thermal cycler for 30 minutes at 20°C with heated lid set to 30°C (or off).

**2. Cleanup of End Repaired DNA**

2.1. Vortex AMPure XP or SPRIselect Beads to resuspend.

2.2. Add 160 µl (1.6X) of resuspended AMPure XP or SPRIselect Beads to the ligation reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.

2.3. Incubate for up to ~5 minutes at room temperature.

2.4. Put the tube/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (~5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain the DNA targets.

2.5 Add 200 µl of 80% freshly prepared ethanol to the tube/PCR plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.

2.6 Repeat Step 2.5 once for a total of two washes.

2.7. Air dry beads for up to 5 minutes while the tube/PCR plate is on the magnetic stand with the lid open.

**Caution: Do not over-dry the beads. This may result in lower recovery of DNA target. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack they are too dry.**

2.8 Remove the tube/plate from the magnet. Elute the DNA target from the beads by adding 47 µl of 10 mM Tris-HCl or 0.1 X TE.

2.9 Mix well on a vortex mixer or by pipetting up and down 10 times and incubate for 2 minutes at room temperature.

2.10. Put the tube/PCR plate in the magnetic stand until the solution is clear. Without disturbing the bead pellet, carefully transfer 42 µl of the supernatant to a fresh, sterile microfuge tube.

## Kit Components

### NEB #E6050S Table of Components

| NEB # | PRODUCT | VOLUME |
|---|---|---|
| E6051A | NEBNext End Repair Enzyme Mix | 0.1 ml |
| E6052A | NEBNext End Repair Reaction Buffer | 0.2 ml |

### NEB #E6050L Table of Components

| NEB # | PRODUCT | VOLUME |
|---|---|---|
| E6051AA | NEBNext End Repair Enzyme Mix | 0.5 ml |
| E6052AA | NEBNext End Repair Reaction Buffer | 1.0 ml |

## Revision History

| REVISION # | DESCRIPTION | DATE |
|---|---|---|
| 1.1 | | 3/12 |
| 2.0 | Create "Kit Component – Table of Components" for small and large size kits. Delete individual component information pages | 4/18 |
| 3.0 | Add "Designed for Use", "Materials not Included". Update the introduction text and the protocol. | 2/19 |
| 4.0 | New format applied. | 1/20 |
| 5.0 | Update protocol and required materials not included | 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