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The NEBNext Multiplex Oligos for Illumina (Index Primers Set 4) Includes:

The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E7730S) and 96 reactions (NEB #E7730L). All reagents should be stored at –20°C.

NEBNext Adaptor for Illumina
USER Enzyme
NEBNext Universal PCR Primer for Illumina
NEBNext Index 30 Primer for Illumina
NEBNext Index 37 Primer for Illumina
NEBNext Index 38 Primer for Illumina
NEBNext Index 39 Primer for Illumina
NEBNext Index 40 Primer for Illumina
NEBNext Index 41 Primer for Illumina
NEBNext Index 43 Primer for Illumina
NEBNext Index 44 Primer for Illumina
NEBNext Index 45 Primer for Illumina
NEBNext Index 46 Primer for Illumina
NEBNext Index 47 Primer for Illumina
NEBNext Index 48 Primer for Illumina
Required Materials Not Included for DNA or ChIP Libraries:

Enzymes and buffers appropriate for DNA or ChIP Illumina library preparation. (DNA: #E7645, #E7370, #E6040, #E6000    ChIP: #E7645, #E7370, #E6240, #E6200)
Agencourt® AMPure® XP Beads (Beckman Coulter®, Inc. #A63881)
Magnetic Stand
DNA LoBind® Tubes (Eppendorf®)/PCR Tubes/96-well PCR Plates
Freshly prepared 80% Ethanol
Nuclease-free Water
0.1X TE (or 10 mM Tris-HCl, pH 7.5–8.0)
10 mM Tris-HCl, pH 8.0
10 mM NaCl
Bioanalyzer® (Agilent Technologies®, Inc.)

Required Materials Not Included for RNA Libraries:

Enzymes and buffers appropriate for RNA Illumina library preparation. (#E7420, #E7530, #E6110, #E6100)
NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB #E7490), or NEBNext rRNA Depletion Kit (Human, Mouse, Rat) (NEB #E6310) or similar
Magnetic Rack (Alpaqua, cat #A001322 or equivalent)
80% Ethanol (freshly prepared)
0.1X TE (or 10 mM Tris-HCl, pH 7.5–8.0)
10 mM NaCl
Agencourt AMPure XP Beads (Beckman Coulter, Inc. #A63881)
Actinomycin D (Sigma# A1410, dissolved in dimethylsulfoxide [DMSO] to 5 μg/μl).
DNA LoBind Tubes/PCR Tubes/96-well PCR Plates
Bioanalyzer (Agilent Technologies, Inc.)

Additional materials required for use with #E6110 and #E6100:
3 M Sodium Acetate, pH 5.5
100% Ethanol
PCR Column Purification Kit (Qiagen® or other)
DNA Gel Extraction Column Purification Kit
RNeasy® MinElute® Clean Up Kit (Qiagen #74204)
Applications:
The NEBNext Multiplex Oligos for Illumina (Index Primers Set 4) contains adaptors and primers that are ideally suited for multiplex sample preparation for next-generation sequencing on the Illumina platform (Illumina, Inc.). Each of these components must pass rigorous quality control standards and is lot controlled, both individually and as a set of reagents.

Lot Control: The lots provided in the NEBNext Multiplex Oligos for Illumina (Index Primers Set 4) are managed separately and are 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 genomic DNA libraries as well as human mRNA libraries and ChIP seq libraries on the Illumina 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.
Figure 1: Workflow demonstrating the use of NEBNext Multiplex Oligos for Illumina (Index Primers Set 4)
Please Refer to the Kit Specific Protocol for using the NEBNext Multiplex Oligos for Illumina:

The following kits are designed for use with the NEBNext Multiplex Oligos for Illumina:

- #E7645, NEBNext Ultra II DNA Library Prep Kit for Illumina
- #E7595, NEBNext Ultra II Ligation Module
- #E7420, NEBNext Ultra Directional RNA Library Prep Kit for Illumina
- #E7530, NEBNext Ultra RNA Library Prep Kit for Illumina
- #E7370, NEBNext Ultra DNA Library Prep Kit for Illumina
- #E7445, NEBNext Ultra Ligation Module
- #E6040, NEBNext DNA Library Prep Master Mix Set for Illumina
- #E6110, NEBNext mRNA Library Prep Master Mix Set for Illumina
- #E6240, NEBNext ChIP-Seq Library Prep Master Mix Set for Illumina
- #E6056, NEBNext Quick Ligation Module
- #E6000, NEBNext DNA Library Prep Reagent Set for Illumina
- #E6200, NEBNext ChIP-Seq Library Prep Reagent Set for Illumina
- #E6100, NEBNext mRNA Library Prep Reagent Set for Illumina
NEBNext Adaptor for Illumina

#E7337A: 0.24 ml  Concentration: 15 µM
#E7337AA: 0.96 ml

5´-/5Phos/GAT CGG AAG AGC ACA CGT CTG AAC TCC AGT C/ideoxyU/A CAC TCT TTC CCT ACA CGA CGC TCT TCC GAT C*T-3´

Store at –20°C

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing this adaptor and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing this reaction buffer at 1X concentration and 1 µg T3 DNA incubated for 16 hours at 37°C also results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

Endonuclease Activity: Incubation of a minimum of 5 µl of this adaptor with 1 µg of φX174 RF 1 DNA in assay buffer for 4 hours at 37°C in 50 µl reactions results in < 10% conversion to RF II as determined by agarose gel electrophoresis.

Phosphatase Activity: Incubation of a minimum of 10 µl of this adaptor in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl₂) containing 2.5 mM p-nitrophenyl phosphate at 37°C for 4 hours yields no detectable p-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

RNase Activity: Incubation of this adaptor with 40 ng of a FAM-labeled RNA transcript for 16 hours at 37°C results in no detectable RNase activity as determined by polyacrylamide gel electrophoresis.

Lot Controlled
**USER Enzyme**

#E7338A: 0.072 ml  
#E7338AA: 0.288 ml

Store at –20°C

Supplied in: 50 mM KCl, 5 mM NaCl, 10 mM Tris-HCl (pH 7.4 @ 25°C), 0.1 mM EDTA, 1 mM DTT, 175 µg/ml BSA and 50% Glycerol

**Quality Control Assays**

**Non-Specific DNase Activity (16 Hour):** A 50 µl reaction in NEBuffer 1 containing 1 µg of Lambda DNA and a minimum of 50 units of Uracil DNA Glycosylase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. A 50 µl reaction in Endonuclease VIII Reaction Buffer containing 1 µg of Lambda-HindIII DNA and a minimum of 25 units of Endonuclease VIII incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**Exonuclease Activity (Radioactivity Release):** A 50 µl reaction in NEBuffer 1 containing 1 µg of a mixture of single and double-stranded [³H] E. coli DNA and a minimum of 50 units of Uracil DNA Glycosylase incubated for 4 hours at 37°C releases < 0.1% of the total radioactivity. A 50 µl reaction in Endonuclease VIII Reaction Buffer containing 1 µg of a mixture of single and double-stranded [³H] E. coli DNA and a minimum of 10 units of Endonuclease VIII incubated for 4 hours at 37°C releases < 0.5% of the total radioactivity.

**Endonuclease Activity (Nicking):** A 50 µl reaction in UDG Reaction Buffer containing 1 µg of supercoiled φX174 DNA and a minimum of 50 units of Uracil DNA Glycosylase incubated for 4 hours at 37°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

**Phosphatase Activity:** Incubation of a minimum of 10 µl of USER at a 1X concentration in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl₂) containing 2.5 mM p-nitrophenyl phosphate at 37°C for 4 hours yields no detectable p-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

Lot Controlled
NEBNext Universal PCR Primer for Illumina

#E6861A: 0.120 ml  Concentration: 10 µM
#E6861AA: 0.480 ml

5´-AAT GAT ACG GCG ACC ACC GAG ATC TAC ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC*T-3´

Store at –20°C

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing this primer and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing 1 µl primer and 1 µg T3 DNA incubated for 16 hours at 37°C also results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

Endonuclease Activity: Incubation of a minimum of 5 µl of primer with 1 µg of φX174 RF I DNA in assay buffer for 4 hours at 37°C in 50 µl reactions results in < 10% conversion to RF II as determined by agarose gel electrophoresis.

RNase Activity: Incubation of 1 µl of primer with 40 ng of a FAM-labeled RNA transcript for 16 hours at 37°C results in no detectable RNase Activity as determined by polyacrylamide gel electrophoresis.

Phosphatase Activity: Incubation of a minimum of 10 µl of this primer in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl₂) containing 2.5 mM p-nitrophenyl phosphate at 37°C for 4 hours yields no detectable p-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

Lot Controlled
**NEBNext Index Primers for Illumina**

**Description:** 12 Index Primers are included for producing barcoded libraries.

<table>
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<tr>
<th>NEB #</th>
<th>PRODUCT</th>
<th>INDEX PRIMER SEQUENCE</th>
<th>EXPECTED INDEX PRIMER SEQUENCE READ</th>
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<td>NEBNext Index 38 Primer for Illumina</td>
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<td>TGG CCA</td>
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</table>

Where -s- indicates phosphorothioate bond.

Note: If fewer than 12 indexes are used in a lane for sequencing, it is recommended to use the following indices:

- Pool of 2 samples: Index #37 and 45
- Pool of 3 samples: Index #30, 38 and 48
- Pool of 4 samples: Index #39, 43, 44 and 46
  (other combinations are possible for pooling > 2 samples)
NEBNext Index Primers for Illumina (Cont.)

Store at –20°C  Concentration: 10 µM

Quality Control Assays

**16-Hour Incubation:** 50 µl reactions containing 1 µl NEBNext Index [X] Primer and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing NEBNext Index [X] Primer for Illumina and 1 µg of T3 DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

**endonuclease Activity:** Incubation of a 50 µl reaction containing 1 µl NEBNext Index [X] Primer with 1 µg of φX174 RF I supercoiled DNA for 4 hours at 37°C results in less than 10% conversion to RF II (nicked molecules) as determined by agarose gel electrophoresis.

**RNase Activity:** Incubation of a 10 µl reaction containing 1 µl NEBNext Index [X] Primer with 40 ng of RNA transcript for 16 hours at 37°C resulted in no detectable degradation of RNA as determined by gel electrophoresis.

**Phosphatase Activity:** Incubation of NEBNext Index [X] Primer in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM MgCl₂) containing 2.5 mM p-nitrophenyl phosphate at 37°C for 4 hours yields no detectable p-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

**Lot Controlled**
Revision History:

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