SAMPLE PREPARATION

NEBNext® mRNA Library Prep Master Mix Set for 454™

Instruction Manual

NEB #E6116S/L
10/50 reactions

NEW ENGLAND BioLabs® Inc.
enabling technologies in the life sciences

NEBNext mRNA Library Prep Master Mix Set for 454

Table of Contents:

Cloned at NEBiolabs | Recombinant Enzyme | NEB2 Optimum Buffer | BSA Requires BSA | Yes Heat Inactivation

The Master Mix Set Includes:

The volumes provided are sufficient for preparation of up to 10 reactions (NEB #E6116S) and 50 reactions (NEB #E6116L). (All reagents should be stored at –20°C).

NEBNext RNA Fragmentation Buffer (10X)
NEBNext RNA Fragmentation Stop Solution (10X)
Linear Acrylamide (10 mg/ml)
Random Primers (3 µg/µl)
Murine RNase Inhibitor RR
NEBNext First Strand Synthesis Reaction Buffer (5X)
NEBNext Second Strand Synthesis Enzyme Mix
NEBNext Second Strand Synthesis Reaction Buffer (10X)
NEBNext End Prep Enzyme Mix
NEBNext End Repair Reaction Buffer (10X)
Quick T4 DNA Ligase RR
NEBNext Sizing Buffer
TE Buffer
Nuclease-free water

Required Materials Not Included:

3 M Sodium Acetate, pH 5.5
100% Ethanol
70% Ethanol
SuperScript® II Reverse Transcriptase (Life Technologies, Inc.)
PCR Column Purification Kit (Qiagen or other)
RNeasy® MinElute® Columns (Qiagen or other)
Agencourt AMPure® Beads (Beckman Coulter, Inc.)
DNA Adaptors and Primers or Vector
Magnetic Separation Rack (NEB #S1510)

Applications:

The NEBNext mRNA Library Prep Master Mix Set for 454 contains enzymes and buffers that are ideally suited for sample preparation for next-generation sequencing, and for preparation of expression libraries. Each of these components must pass rigorous quality control standards and are Lot Controlled, both individually and as a set of reagents.

Lot Control: The lots provided in the NEBNext mRNA Library Prep Master Mix Set for 454 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 a transcriptome library with 454 GS FLX Titanium™ System (Roche).

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.

Protocols:

Starting Material: 100–200 ng purified mRNA, 200 ng recommended.

mRNA Fragmentation Protocol

1. Mix the following components in a sterile PCR tube:

Purified mRNA	1–18 µl
10X RNA Fragmentation Reaction Buffer	2 µl
Nuclease-Free Water	variable
Total volume	20 µl

2. Incubate in a preheated thermal cycler for 1–5 minutes at 94°C. Fragmentation time should be adjusted depending on type of RNA and desired sizes of fragments (see Figure 1).
3. Transfer tube to ice.
4. Add 2 µl 10X RNA Fragmentation Stop Solution.

Clean Up Fragmented RNA Using RNeasy MinElute Spin Columns

1. Add 78 µl of the Nuclease-Free Water to the 22 µl fragmented RNA from step 4. Purify sample using RNeasy MinElute Cleanup Kit (Qiagen #74204) following manufacture instructions. Elute in 15.5 µl Nuclease-Free Water. The recovered volume should be ~14.5 µl.

   Note: column purification removes short RNA Fragments and enriches the sample for RNA fragments longer than 200 nucleotides.

Alternatively, Clean Up Fragmented RNA Using Ethanol Precipitation

1. Mix the following components in a sterile 1.5 ml microcentrifuge tube:

	Volume (µl)
Fragmented RNA from Step 4	22
3M Sodium Acetate, pH 5.5	2
Linear Acrylamide, 10 mg/ml	1–2
100% Ethanol	60
total volume	85–86

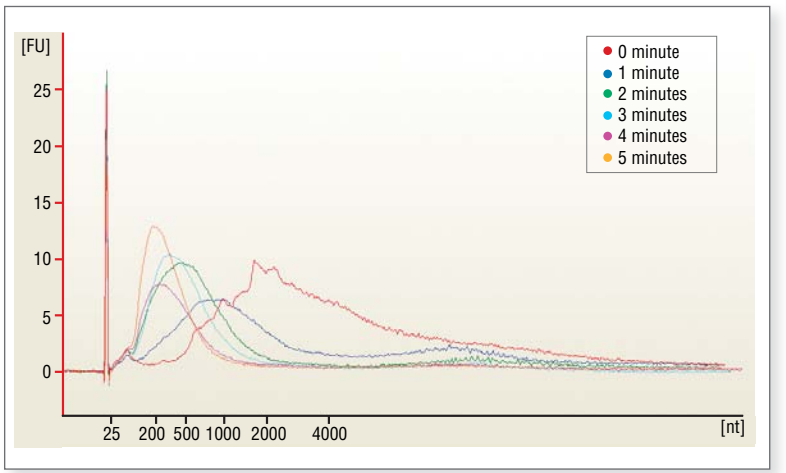
2. Incubate at –80°C for 30 minutes.
3. Centrifuge at 14,000 rpm for 25 minutes at 4°C in a microcentrifuge.
4. Carefully remove ethanol.
5. Wash pellet with 300 µl of 70% ethanol.

6. Centrifuge and carefully remove 70% ethanol.
7. Air dry pellet for up to 10 minutes at room temperature to remove residual ethanol.
8. Resuspend in 14.5 µl Nuclease-Free Water.

Assess the Yield and the Size Distribution of the Fragmented RNA.

Run 1 µl in the Agilent Bioanalyzer® 2100 (Agilent Technologies, Inc.) using a RNA Pico chip (Figure 1).

Figure 1: Relative size distribution of eukaryotic mRNA fragments as seen using the Bioanalyzer 2100.



Poly (A)+ mRNA (40 ng) purified from the Universal Human Reference Total RNA (Stratagene) was fragmented in 1X NEBNext Magnesium RNA Fragmentation Buffer for 1–5 minutes at 94°C. Fragmentation Reaction was stopped in 1X NEBNext Fragmentation Stop Solution. Samples were diluted 1:10 in Nuclease-Free Water and analyzed in the Bioanalyzer 2100.

First Strand cDNA Synthesis

1. Mix the following components in a sterile PCR tube:

Fragmented mRNA	13.5 µl
Random Primers	1 µl
Total volume	14.5 µl

2. Incubate in a preheated thermal cycler for 5 minutes at 65°C.
3. Spin tube briefly and place on ice
4. To the fragmented mRNA and Random Primers add:

5X First Strand Synthesis Reaction Buffer	4 µl
Murine RNase Inhibitor	0.5 µl
Total volume	19 µl

5. Incubate in a preheated thermal cycler for 2 minutes at 25°C.
6. Add 1 µl SuperScript II Reverse Transcriptase to the reaction.
7. Incubate sample as follows:
   10 minutes at 25°C
   50 minutes at 42°C
   15 minutes at 70°C
   Hold at 4°C
8. Place the tube on ice.

Second Strand cDNA Synthesis

1. Add 48 µl Nuclease-Free Water to the First Strand Synthesis reaction.
2. To the reaction then add:

10X Second Strand Synthesis Reaction Buffer	8 µl
Second Strand Synthesis Enzyme Mix	4 µl
Total volume	80 µl

3. Mix thoroughly by gentle pipetting.
4. Incubate in a thermal cycler for 2.5 hours at 16°C.
5. Purify cDNA using a PCR column purification kit, purifying the sample on one column and eluting in 16 µl sterile water or elution buffer.

NEBNext End Repair and dA-Tailing

1. In a 1.7 ml micro-centrifuge tube add:

End Prep Enzyme Mix	1.0 µl
End Repair Reaction Buffer (10X)	2.5 µl
Nuclease -free Water	5.5 µl
	9.0 µl

2. Mix by pipetting and add to the 16 µl purified, double stranded cDNA.
3. Vortex briefly to mix, followed by a quick spin to collect all liquid from the sides of the tube.
4. In a thermocycler, with the heated lid on, run the following program:
   20 minutes at 25°C
   20 minutes at 72°C
   Hold at 4°C

Agencourt AMPure Beads Preparation

1. Vortex AMPure beads to re-suspend.
2. Transfer 125 µl of AMPure beads to a 1.7 ml micro-centrifuge tube.
3. Place the tube on a Magnetic Separation Rack. After the beads have collected to the side of the tube and the solution is clear, carefully remove and discard the supernatant. Be careful not to disturb the beads.
4. Add 73 µl of TE to the beads and vortex until the beads are completely re-suspended.
5. Add 500 µl of NEBNext Sizing Buffer to the beads, vortex to mix and spin briefly to collect all liquid from the sides of the tube.

NEBNext Adaptor Ligation

1. After completion of the End Repair/dA-Tailing step, add 1.0 µl of adaptor (or vector if generating an expression library), to the reaction tube. (Adaptors/vectors are not provided. Please use adaptors/vectors appropriate to specific application).
2. Add 1.0 µl of Quick T4 DNA Ligase. Vortex briefly to mix, followed by a quick spin.
3. Incubate for 10 minutes at 25°C.

NEBNext Small Fragment Removal

1. Add the End Repaired/dA-Tailed/Adaptor ligated DNA sample directly to the previously prepared Ampure beads. Vortex briefly to mix, followed by a quick spin to collect liquid from the sides of the tube.
2. Incubate at room temperature for 5 minutes.
3. Place the tube on a Magnetic Separator.
4. When the beads have collected to the wall of the tube and the solution is clear, remove and discard the supernatant. Be careful not to disturb the beads.
5. Add 100 µl of TE and vortex until the beads are completely re-suspended.
6. Add 500 µl of NEBNext Sizing Buffer and briefly vortex to mix.
7. Incubate at room temperature for 5 minutes.
8. Place the tube on a Magnetic Separator.
9. When the beads are collected to the wall of the tube and the solution is clear, remove and discard the supernatant. Be careful not to disturb the beads.

10. Repeat steps 5-9 one time.

11. Keep the tube on the magnet and wash the beads twice with 1 ml of 70% ethanol.

12. Keep the tube on the magnet, uncapped, and let the pellet air dry until there is no visible liquid on the sides of the tube. This typically takes 5 minutes.

13. Remove the tube from the magnet, add 53 µl of TE, vortex to re-suspend the beads and spin briefly.

14. Place the tube on the magnet, when the beads are collected to the wall of the tube, transfer 50 µl of the supernatant (library), to a new 1.7 ml micro-centrifuge tube. Be careful not to transfer any beads.

NEBNext RNA Fragmentation Buffer

#E6101A: 40 µl **Concentration: 10X**
#E6101AA: 200 µl

Store at –20°C or 4°C

1X NEBNext RNA Fragmentation Buffer:
40 mM Tris-OAc
100 mM KOAc
30 mM $Mg(OAc)_2$
pH 8.3 @ 25°C

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1X RNA Fragmentation Buffer 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 1X RNA Fragmentation Buffer 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 10 µl reaction containing 1X RNA Fragmentation Buffer with 1 µg of ϕX174 RF I supercoiled DNA for 4 hours at 37°C results in < 10% conversion to RF II (nicked molecules) as determined by gel electrophoresis.

Phosphatase Activity: Incubation of 1X RNA Fragmentation Buffer in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) 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 RNA Fragmentation Stop Solution

#E6102A: 40 µl **Concentration: 10X**
#E6102AA: 200 µl

Store at –20°C

1X NEBNext RNA Fragmentation Stop Solution:
50 mM EDTA

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1X RNA Fragmentation Stop Solution 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 1X RNA Fragmentation Stop Solution 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 10 µl reaction containing 1X RNA Fragmentation Stop Solution with 1 µg of ϕX174 RF I supercoiled DNA for 4 hours at 37°C results in < 10% conversion to RF II (nicked molecules) as determined by gel electrophoresis.

RNase Activity: Incubation of a 10 µl reaction containing 1X RNA Fragmentation Stop Solution 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 1X RNA Fragmentation Stop Solution in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) 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

Linear Acrylamide

#E6103A: 20 µl **Concentration: 10 mg/ml**
#E6103AA: 100 µl

Store at –20°C or 4°C

1X Linear Acrylamide:
10 mg/ml Linear Acrylamide in sterile water

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1 µg Linear Acrylamide 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 µg Linear Acrylamide 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 10 µl reaction containing 1 µg Linear Acrylamide with 1 µg of ϕX174 RF I supercoiled DNA for 4 hours at 37°C results in < 10% conversion to RF II (nicked molecules) as determined by gel electrophoresis.

RNase Activity: Incubation of a 10 µl reaction containing 1 µg Linear Acrylamide 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 1 µg Linear Acrylamide in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) 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

Random Primers

#E6104A: 15 µl
#E6104AA: 50 µl

Store at –20°C

Description: This mixture of random hexanucleotides is used to prime DNA synthesis *in vitro* along multiple sites of template RNA.

Sequence: 5´ d(N^6) 3´ [N=A,C,G,T]

Phosphorylated: No.

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1 µl Random Primers 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 Random Primers 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 10 µl reaction containing 1 µl Random Primers with 1 µg of ϕX174 RF I supercoiled DNA for 4 hours at 37°C results in < 10% conversion to RF II (nicked molecules) as determined by gel electrophoresis.

RNase Activity: Incubation of a 10 µl reaction containing 1 µl Random Primers 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 1 µl Random Primers in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) 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

Murine RNase Inhibitor

#E6105A: 15 µl
#E6105AA: 25 µl

Store at –20°C

Description: Murine RNase inhibitor is a 50 kDa recombinant protein of murine origin. The inhibitor specifically inhibits RNases A, B and C. It inhibits RNases by binding noncovalently in a 1:1 ratio with high affinity. It is not effective against RNase 1, RNase T1, S1 Nuclease, RNase H or RNase from *Aspergillus*. In addition, no inhibition of polymerase activity is observed when RNase Inhibitor is used with *Taq* DNA Polymerase, AMV or M-MuLV Reverse Transcriptases, or Phage RNA Polymerases (SP6, T7, or T3).

Recombinant murine RNase inhibitor does not contain the pair of cysteines identified in the human version that is very sensitive to oxidation, which causes inactivation of the inhibitor (1). As a result, murine RNase inhibitor has significantly improved resistance to oxidation compared to the human/porcine RNase inhibitors, even under conditions where the DTT concentration is low. Therefore, it is advantageous to use murine RNase inhibitor in reactions where high concentration DTT is adverse to the reaction (eg. Real-time RT-PCR).

Source: An *E. coli* strain that carries the Ribonuclease Inhibitor gene from mouse.

Supplied in: 20 mM HEPES-KOH, 50 mM KCl, 8 mM DTT and 50% glycerol.

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing a minimum of 40 units of Murine RNase Inhibitor 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 a minimum of 40 units of Murine RNase Inhibitor 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.

Exonuclease Activity: Incubation of a 50 µl reaction containing 200 units of Murine RNase Inhibitor with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA (20^5 cpm/µg) for 4 hours at 37°C released < 0.5% of the total radioactivity.

Latent RNase Assay: Heating the Murine RNase Inhibitor for 20 minutes at 65°C, followed by incubation of a 10 µl reaction containing 40 units of RNase Inhibitor with 40 ng of RNA transcript for 4 hours at 37°C resulted in no detectable degradation of RNA as determined by gel electrophoresis.

RNase Activity: Incubation of a 10 µl reaction containing 40 units of Murine RNase Inhibitor with 40 ng of RNA transcript for 16 hours at 37°C resulted in no detectable degradation of RNA as determined by gel electrophoresis.

Endonuclease Activity: Incubation of a 10 µl reaction containing 40 units of Murine RNase Inhibitor with 1 µg of ϕX174 RF I supercoiled DNA for 4 hours at 37°C results in < 10% conversion to RF II (nicked molecules) as determined by gel electrophoresis.

Phosphatase Activity: Incubation of a minimum of 40 units of Murine RNase Inhibitor in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) 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

References:

1. Kim, B.M. et al. (1999). *Protein Science*, 8, 430–434.

NEBNext First Strand Synthesis Reaction Buffer

#E6106A: 40 µl **Concentration: 5X**
#E6106AA: 200 µl

Store at –20°C

1X NEBNext First Strand Synthesis Reaction Buffer:
50 mM Tris-Acetate
75 mM KOAc
3.1 mM $Mg(OAc)_2$
0.5 mM dNTPs each
pH 8.3 @ 25°C

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1X First Strand Synthesis Reaction Buffer 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 1X First Strand Synthesis Reaction Buffer 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 10 µl reaction containing 1X First Strand Synthesis Reaction Buffer with 1 µg of ϕX174 RF I supercoiled DNA for 4 hours at 37°C results in < 10% conversion to RF II (nicked molecules) as determined by gel electrophoresis.

RNase Activity: Incubation of a 10 µl reaction containing 1X First Strand Synthesis Reaction Buffer 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 1X First Strand Synthesis Reaction Buffer in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) 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 Second Strand Synthesis Enzyme Mix

#E6107A: 40 µl
#E6107AA: 200 µl

Store at –20°C

Description: NEBNext Second Strand Synthesis Enzyme Mix is optimized to conver 10–100 ng of short single-stranded cDNAs to double-stranded cDNAs with Random Primers and NEBNext Second Strand Synthesis Reaction Buffer.

NEBNext Second Strand Synthesis Enzyme Mix:
6,000 units/ml DNA Polymerase I (*E. coli*)
5,000 units/ml RNase H
25,000 units/ml *E. coli* DNA Ligase

Supplied in: 10 mM Tris-HCl (pH 7.5 @ 25°C), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA and 50% glycerol.

Quality Control Assays

SDS-PAGE Purity: SDS-PAGE analysis of this enzyme indicates > 95% enzyme purity.

Endonuclease Activity: Incubation of a 10 µl reaction containing 1 µl Second Strand Synthesis Enzyme Mix with 1 µg of ϕX174 RF I supercoiled DNA for 4 hours at 37°C results in < 10% conversion to RF II (nicked molecules) as determined by gel electrophoresis.

Phosphatase Activity: Incubation of a minimum of 1 µl Second Strand Synthesis Enzyme Mix in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) 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.

Functional Activity: One unit of the *E. coli* DNA Ligase ligated 50% of HindIII fragments of λ DNA (5´ DNA termini concentration of 0.12 µM, 300 µg/ml) in a total reaction volume of 20 µl in 30 minutes at 16°C in 1X *E. coli* DNA Ligase Reaction Buffer. One unit of *E. coli* DNA Polymerase I incorporated 10 nmol of dNTP into acid-insoluble material in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X EcoPol Reaction Buffer with 33 µM dNTPs including [^{3}H]-dTTP and 70 µg/ml denatured herring sperm DNA. Incubation of 50 units of RNase H with 1 µg sonicated and denatured [^{3}H]-DNA (10^5 cpm/µg) for 30 minutes at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

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NEBNext Second Strand Synthesis Reaction Buffer

#E6108A: 80 µl **Concentration: 10X**
#E6108AA: 400 µl

Store at –20°C

1X NEBNext Second Strand Synthesis Reaction Buffer:
20 mM Tris-HCl
12 mM $(NH_4)_2SO_4$
5 mM $MgCl_2$
0.16 mM β-NAD
0.19 mM dNTPs each
pH 7.4 @ 25°C

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1X NEBNext Second Strand Synthesis Reaction Buffer 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 1X Second Strand Synthesis Reaction Buffer 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 10 µl reaction containing 1X NEBNext Second Strand Synthesis Reaction Buffer with 1 µg of ϕX174 RF I supercoiled DNA for 4 hours at 37°C results in < 10% conversion to RF II (nicked molecules) as determined by gel electrophoresis.

RNase Activity: Incubation of a 10 µl reaction containing 1X NEBNext Second Strand Synthesis Reaction Buffer 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 1X NEBNext Second Strand Synthesis Reaction Buffer in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) 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.

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NEBNext End Prep Enzyme Mix

#E6091A: 0.015 ml
#E6091AA: 0.075 ml

Store at –20°C

Description: NEBNext End Prep Enzyme Mix is optimized to convert 0.5 µg of fragmented to repaired DNA having 5´-phosphorylated, dA-tailed ends.

NEBNext Quick Master Mix:
1,250 units/ml *Taq* DNA Polymerase
10,000 units/ml T4 Polynucleotide Kinase
3,000 units/ml T4 DNA Polymerase

Storage Conditions:
10 mM Tris-HCl
100 mM KCl
1 mM DTT
0.1 mM EDTA
50% Glycerol
0.1% Triton X-100
pH 7.4 @ 25°C

Quality Control Assays

SDS-PAGE Purity: SDS-PAGE analyses of each individual enzyme indicates > 95% enzyme purity.

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

Phosphatase Activity: Incubation of a minimum of 10 µl of this enzyme mix in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) 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.

Functional Activity (Nucleotide Incorporation, Phosphorylation and dA-Tailing): 1 µl of this enzyme mix repairs and phosphorylates the ends of > 95% of 0.5 µg of DNA fragments containing both 3´ and 5´ overhangs within 20 minutes at 25°C, in 1X End Repair Reaction Buffer, as determined by capillary electrophoresis.

1 µl of this enzyme mixture adds a single nucleotide to the 3´ end of 0.5 µg of repaired DNA fragments within 20 mintues at 72°C in 1X End Repair Reaction Buffer, as determined by capillary electrophoresis.

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NEBNext End Repair Reaction Buffer

#E6092A: 0.025 ml **Concentration: 10X**
#E6092AA: 0.125 ml

Store at –20°C

1X NEBNext End Repair Reaction Buffer:
50 mM Tris-HCl
10 mM $MgCl_2$
10 mM DTT
1 mM ATP
0.4 mM dATP
0.4 mM dCTP
0.4 mM dGTP
0.4 mM dTTP
pH 7.5 @ 25°C

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing this reaction buffer at 1X concentration 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 this reaction buffer at a 1X concentration with 1 µg of ϕX174 RF I DNA for 4 hours at 37°C in 50 µl reactions results in less than 10% conversion to RF II as determined by agarose gel electrophoresis.

RNase Activity: Incubation of this reaction buffer at 1X concentration 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 this reaction buffer at a 1X concentration in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) 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.

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Quick T4 DNA Ligase

#E6087A: 0.015 ml
#E6087AA: 0.075 ml

Store at –20°C

Source: Purified from *E. coli* C600 pcI857 pPLc28 lig8 (2).

Quality Control Assays

SDS-PAGE Purity: SDS-PAGE analysis of this enzyme indicates > 95% enzyme purity.

16-Hour Incubation: 50 µl reactions containing a minimum of 2,000 units of this enzyme 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 a minimum of 2,000 units of this enzyme 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 3,200 units of this enzyme with 1 µg of ϕX174 RF I DNA in assay buffer for 4 hours at 37°C in 50 µl reactions results in less than 10% conversion to RF II as determined by agarose gel electrophoresis.

Phosphatase Activity: Incubation of a minimum of 20,000 units of this enzyme in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) 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 a minimum of 2,000 units of this enzyme 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.

Exonuclease Activity: Incubation of a minimum of 3,200 units of this enzyme with 1 µg sonicated [^{3}H] DNA (10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer releases < 0.1% radioactivity.

Functional Activity (Blunt End Ligation): 50 µl reactions containing a 0.5 µl Quick T4 DNA Ligase, 18 µg HaeIII digested ϕX174 and 1X T4 DNA Ligase Buffer incubated at 16°C for 7.5 min results in > 95% of fragments ligated as determined by agarose gel electrophoresis.

Functional Activity (Cohesive End Ligation): 20 µl reactions containing 0.5 µl Quick T4 DNA Ligase, 12 µg HindIII digested lambda DNA and 1X T4 DNA Ligase Buffer incubated at 37°C overnight results in > 95% of fragments ligated as determined by agarose gel electrophoresis. Redigestion of the ligated products, 50 µl reactions containing 6 µg of the ligated fragments, 40 units HindIII, and 1X NEBuffer 2 incubated at 37°C for 2 hours, results in no detectable undigested fragments as determined by agarose gel electrophoresis.

Functional Activity (Adaptor Ligation): 50 µl reactions containing 0.125 µl Quick T4 DNA Ligase, 8 nmol 12 bp adaptor, and 1X T4 DNA Ligase Buffer incubated at 16°C overnight results in no detectable unligated adaptor as determined by agarose gel electrophoresis.

Functional Activity (Transformation): After a five-minute ligation of linearized, dephosphorylated LITMUS™ 28 (containing either blunt [EcoRV] or cohesive [HindIII] ends) and a mixture of compatible insert fragments, transformation into chemically competent *E. coli* DH-5 alpha cells yields a minimum of 1 x 10^6 recombinant transformants per µg plasmid DNA.

Lot Controlled

References:

1. Engler, M. J. and Richardson, C. C. (1982). In P. D. Boyer (Ed.), *The Enzymes* Vol. 5, (p. 3). San Diego: Academic Press.
2. Remaut, E., Tsao, H. and Fiers, W. (1983) *Gene,* 22, 103–113.

NEBNext Sizing Buffer

#E6088A: 15 mls **Concentration: 1.2X**
#E6088AA: 75 mls

Store at –20°C

1X NEBNext Sizing Buffer:
7% Polyethylene Glycol 8000
1 M NaCl

Description: NEBNext Sizing Buffer has been optimized for use with AMPure beads to select DNA fragments > 300 bp from a sample of mixed sized DNA fragments.

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing this reaction buffer at 1X concentration 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.

RNase Activity: Incubation of this reaction buffer at 1X concentration 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 this reaction buffer at a 1X concentration in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) 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.

Functional Assay (Size Selection): Two rounds of selection, using 500 µl of this buffer and 125 µl of Agencourt Ampure beads, results in the selection of DNA Fragments > 300 bp from 0.5 µg of 100 kb DNA ladder (NEB# N3231), as determined by Agilent Bioanalyzer analysis.

Lot Controlled

TE Buffer

#E6089A: 5 ml **Concentration: 1X**
#E6089AA: 25 ml

Store at –20°C

Description: TE Buffer is free of detectable DNA and RNA nucleases and is suitable for use in molecular biology applications.

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing this reaction buffer at 1X concentration 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 this reaction buffer at a 1X concentration with 1 µg of ϕX174 RF I DNA for 4 hours at 37°C in 50 µl reactions results in less than 10% conversion to RF II as determined by agarose gel electrophoresis.

RNase Activity: Incubation of this reaction buffer at 1X concentration 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 this reaction buffer at a 1X concentration in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) 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

Nuclease-free Water

#E6109A: 4 ml
#E6109AA: 20 ml

Store at –20°C or 4°C

Description: Nuclease-free Water is free of detectable DNA and RNA nucleases and phosphatases and suitable for use in DNA and RNA applications.

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing Nuclease-free Water 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 Nuclease-free Water 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 10 µl reaction containing Nuclease-free Water with 1 µg of ϕX174 RF I supercoiled DNA for 4 hours at 37°C results in < 10% conversion to RF II (nicked molecules) as determined by gel electrophoresis.

RNase Activity: Incubation of a 10 µl reaction containing Nuclease-free Water 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 1X Second Strand Synthesis Reaction Buffer in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) 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

Notes

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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) 837-2234
Toll Free: 1-800-387-1095
Fax: (905) 837-2994
Fax Toll Free: 1-800-563-3789
e-mail: info@ca.neb.com

China, People's Republic
New England Biolabs (Beijing), Ltd.
Telephone: 010-82378265/82378266
Fax: 010-82378262
e-mail: info@neb-china.com

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

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

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

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

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