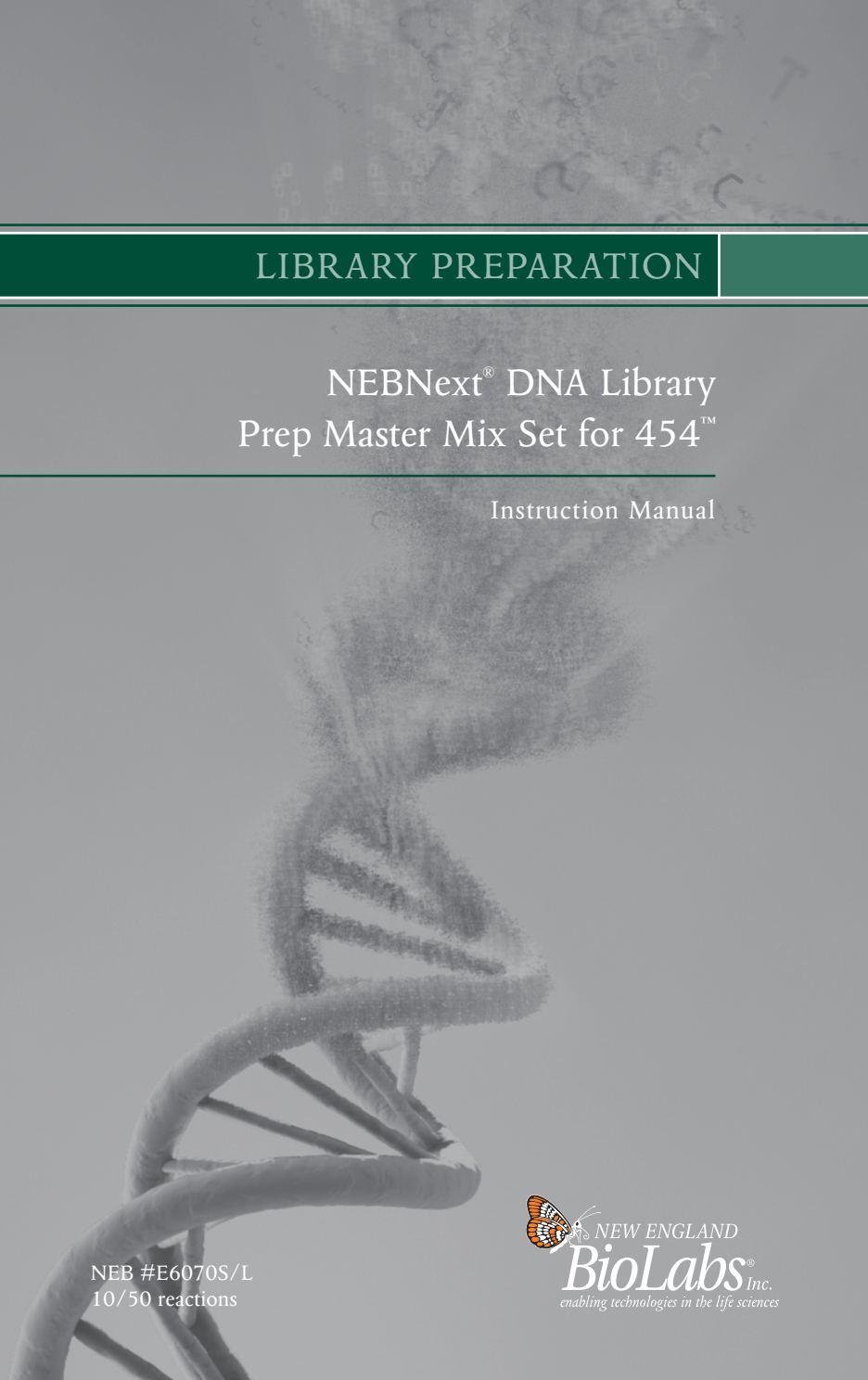
LIBRARY PREPARATION

NEBNext® DNA Library Prep Master Mix Set for 454™

Instruction Manual



NEB #E6070S/L
10/50 reactions

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


Cloned at NEBiolabs


Recombinant Enzyme


Heat Inactivation

NEBNext DNA Library Prep Master Mix Set for 454

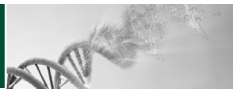


Table of Contents:

The Reagent Set Includes:

The volumes provided are sufficient for preparation of up to 10 reactions (NEB #E6070S) and 50 reactions (NEB #E6070L).

Box 1: Store at –20°C

NEBNext End Repair Enzyme Mix RR

NEBNext End Repair Reaction Buffer (10X)

Quick T4 DNA Ligase RR

NEBNext Quick Ligation Reaction Buffer (5X) RR

Bst DNA Polymerase, Large Fragment RR

NEBNext Adaptor Fill-in Reaction Buffer (10X) RR

Molecular Biology Grade Water RR

Box 2: Store at 4°C

Hydrophilic Streptavidin Magnetic Beads (4 mg/ml)

NEBNext Bead Binding Buffer (2X)

NEBNext Bead Wash Buffer (1X)

Applications:

The NEBNext DNA Library Prep Master Mix Set for 454 contains enzymes and buffers in convenient master mix formulations that are ideally suited for sample preparation for next-generation sequencing (1), and for preparation of single stranded DNA for use in high density hybridization arrays (2) or for genomic subtraction hybridization methods (3). 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 DNA 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.

Functional Validation: Each set of reagents is functionally validated together through construction and sequencing of a genomic DNA library by 454 GS FLX Titanium™ (Roche), and by generation of single stranded DNA.

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.

References:

1. Maricic, T. and S. Paabo (2009). "Optimization of 454 sequencing library preparation from small amounts of DNA permits sequence determination of both DNA strands." *Biotechniques,* 46, 51–52, 54–57.
2. Straus, D. and F.M. Ausubel (1990). "Genomic subtraction for cloning DNA corresponding to deletion mutations." *Proc. Natl. Acad. Sci. USA* 87, 1889–1893.
3. Zhou, X. and D.T. Wong (2007). "Single nucleotide polymorphism mapping array assay." *Methods Mol. Biol.* 396, 295–314.

Protocols:

NEBNext End Repair Module Protocol

Starting Material: 1–5 µg of DNA Fragmented to 100–1000 bp in ≤ 85 µl

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

Fragmented DNA	1–85 µl
NEBNext End Repair Reaction Buffer (10X)	10 µl
NEBNext End Repair Enzyme Mix	5 µl
Sterile H_2O for a final volume of 100 µl	variable
total volume	100 µl

2. Incubate in a thermal cycler for 30 minutes at 20°C.
3. Purify DNA sample on one column and elute in 30 µl of sterile dH_2O or elution buffer.

NEBNext Quick Ligation Module Protocol

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

End Repaired, Blunt or dA-Tailed DNA	30 µl
Quick Ligation Reaction Buffer (5X)	10 µl
DNA Adaptors (not provided please use adaptors appropriate to specific application)	5 µl
Quick T4 DNA Ligase	5 µl
total volume	50 µl

2. Incubate in a thermal cycler for 15 minutes at 20°C.
3. Purify DNA sample on one column and elute in 25 µl of sterile dH_2O or elution buffer.

NEBNext Fill-in and ssDNA Isolation Module Protocol

Recommended: Removal of small fragments using Agencourt AMPure® Beads (Beckman Coulter, Inc.) or gel size selection.

1. Transfer 50 µl of Hydrophilic Streptavidin Magnetic Beads to a 1.5 ml tube.
2. Using a magnet, pellet the beads and remove the buffer
3. Wash beads twice with 100 µl of 2X Bead Binding Buffer, pelleting the beads with a magnet to remove the buffer after each wash.
4. Resuspend beads in 25 µl of 2X Bead Binding Buffer.
5. Add 25 µl adapter-ligated DNA fragments to the beads.
6. Vortex and place on a tube rotator at room temperature for 20 minutes.
7. Using a magnet, wash the beads twice with 100 µl (1X) Bead Wash Buffer, pelleting the beads with a magnet to remove the buffer after each wash.

8. Mix the following components in a separate sterile microfuge tube:

Molecular Biology Grade Water	42 µl
Adapter Fill-in Reaction Buffer	5 µl
Bst DNA Polymerase, Large Fragment	3 µl

9. Transfer the 50 µl Fill-in Reaction Mix to the beads.
10. Vortex lightly and incubate at 37°C for 20 minutes.
11. Wash beads twice with 100 µl of 1X Bead Wash Buffer, pelleting the beads with a magnet to remove the buffer after each wash.
12. Prepare Melt Solution:

10 N NaOH	125 µl
Water	9.875 ml

13. Prepare Neutralization Solution in a 1.5 ml tube.

3 M sodium acetate, pH 5.2	10 µl
Column binding buffer with pH indicator	500 µl

14. Add 50 µl of Melt Solution to the beads.
15. Vortex well, pellet the beads with a magnet.
16. Carefully transfer the Melt Solution containing the ssDNA Fragment Library to 1.5 ml tube containing the Neutralization Solution.
17. Repeat steps 14–16, adding the second round of Melt Solution containing the ssDNA Fragment Library to the same 1.5 ml tube containing the Neutralization Solution and the first round of Melt Solution and ssDNA Fragments. Adjust pH if necessary by adding an additional 5 µl of 3 M sodium acetate.
18. Purify DNA on one column without adding any additional column binding buffer. Wash column twice with column wash buffer to remove all residual salts. Elute in 25 µl of sterile dH_2O or elution buffer.

NEBNext End Repair Enzyme Mix

#E6041A: 0.06 ml
#E6041AA: 0.3 ml

Store at –20°C

Description: NEBNext End Repair Enzyme Mix is optimized to convert 1 to 5 µg of fragmented to repaired DNA having 5´-phosphorylated, blunt ends.

NEBNext End Repair Enzyme Mix:
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): 0.2 µl of this enzyme mix incorporates 10 nmol of dNTP into acid-precipitable material in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X T4 DNA Polymerase Reaction Buffer with 33 µM dNTPs including [^{3}H]-dTTP, 70 µg/ml denatured herring sperm DNA and 50 µg/ml BSA.

Functional Activity (Nucleotide Incorporation and Phosphorylation): 5 µl of this enzyme mix repairs and phosphorylates the ends of > 95% of 10 µg of DNA fragments containing both 3´ and 5´ overhangs within 30 minutes at 20°C in 1X End Repair Buffer, as determined by capillary electrophoresis.

Lot Controlled

NEBNext End Repair Reaction Buffer

#E6042A: 0.12 ml **Concentration: 10X**
#E6042AA: 0.6 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 HaeIII digested ϕX174 RF I 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

Quick T4 DNA Ligase

#E6047A: 0.06 ml
#E6047AA: 0.3 ml

Store at –20°C

Source: Purified from *E. coli* C600 pcl857 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 HaeIII digested ϕX174 RF I 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 (Adapter Ligation): 50 µl reactions containing 0.125 µl Quick T4 DNA Ligase, 8 nmol 12 bp adapter, and 1X T4 DNA Ligase Buffer incubated at 16°C overnight results in no detectable unligated adapter 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 Quick Ligation Reaction Buffer

#E6048A: 0.12 ml **Concentration: 5X**
#E6048AA: 0.6 ml

Store at –20°C

1X NEBNext Quick Ligation Reaction Buffer:
66 mM Tris-HCl
10 mM $MgCl_2$
1 mM dithiothreitol
1 mM ATP
6% Polyethylene glycol (PEG 6000)
pH 7.6 @ 25°C

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing this reaction buffer at 1X concentration and 1 µg of HaeIII digested ϕX174 RF I 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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Bst DNA Polymerase, Large Fragment

#E6030A: 0.03 ml
#E6030AA: 0.15 ml

Store at –20°C

Description: *Bst* DNA Polymerase, Large Fragment is the portion of the *Bacillus stearothermophilus* DNA Polymerase protein that contains the 5´→ 3´ polymerase activity, but lacks 5´→ 3´ exonuclease activity.

Source: *Bst* Polymerase, Large Fragment is prepared from an *E. coli* strain containing a genetic fusion of the *Bacillus stearothermophilus* DNA Polymerase gene, lacking the 5´→ 3´ exonuclease domain, and the gene coding for *E. coli* maltose binding protein (MBP). The fusion protein is purified to near homogeneity and the MBP portion of the fusion is cleaved off *in vitro.* The remaining polymerase is purified free of MBP (1).

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM dithiothreitol, 0.1% Triton X-100 and 50% glycerol.

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 8 units of this enzyme and 1 µg of HaeIII digested ϕX174 RF I 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 8 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 50 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 50 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 8 units of this enzyme with 40 ng of FAM-labeled RNA transcript for 16 hours at 37°C results in no detectable RNase activity as determined by polyacrylamide gel electrophoresis.

Functional Activity (Nucleotide Incorporation): One unit of this enzyme incorporates 10 nmol of dNTP into acid-precipitable material in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X NEBuffer 2 with 33 µM dNTPs including [^{3}H]-dTTP, 70 µg/ml denatured herring sperm DNA and 50 µg/ml BSA.

Lot Controlled

References:

1. Kong, H., Aliotta, J. and Pelletier, J.J., New England Biolabs, unpublished results.

U.S. Patent No. 5,814,506.

NEBNext Adapter Fill-in Reaction Buffer

#E6035A: 0.05 ml **Concentration: 10X**
#E6035AA: 0.25 ml

Store at –20°C

1X NEBNext Adapter Fill-in Reaction Buffer:
20 mM Tris-HCl
10 mM $(NH_4)_2SO_4$
10 mM KCl
2.0 mM $MgSO_4$
0.1% Triton X-100
0.4 mM dATP
0.4 mM dCTP
0.4 mM dGTP
0.4 mM dTTP
pH 8.8 @ 25°C

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing this reaction buffer at 1X concentration and 1 µg of HaeIII digested ϕX174 RF I 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.

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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Molecular Biology Grade Water

#E6031A: 1 ml
#E6031AA: 5 ml

Store at –20°C

Description: Molecular Biology Grade Water is free of detectable DNA and RNA nucleases and phosphatases and suitable for use in molecular biology applications.

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing molecular biology grade water, 1X NEBuffer 2 (50mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.9) and 1 µg of HaeIII digested ϕX174 RF I 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 43 µl of Molecular Biology Grade Water 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 molecular biology grade water, 1X NEBuffer 2 (50mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.9) and 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 molecular biology grade water with 1X NEBuffer 2 (50mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.9) and 40 ng of a FAM-labeled RNA transcript in a 10 µl reaction for 16 hours at 37°C results in no detectable RNase activity as determined by polyacrylamide gel electrophoresis.

Phosphatase Activity: Incubation of molecular biology grade water in protein phosphatase assay buffer (1M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) containing 2.5 mM *p*-nitrophenyl phosphate in a 200 µl reaction at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

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Hydrophilic Streptavidin Magnetic Beads

#E6032A: 0.5 ml **Concentration: 4 mg/ml**
#E6032AA: 2.5 ml

Store at 4°C

Description: Hydrophilic Streptavidin Magnetic Beads are 2 µm supermagnetic particles covalently coupled to a highly pure form of streptavidin. The beads can be used to capture biotin labeled DNA.

Supplied in: 4 mg/ml suspension in phosphate buffer (PBS) (pH 7.4) containing 0.1% BSA and 0.02% NaN_3.

Support Matrix: 2 µm non-porous magnetic microparticle.

Binding Capacity: The beads will bind greater than 800 pmol of free biotin per mg and greater than 400 pmol of single-stranded 20 bp biotinylated oligonucleotide per mg.

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NEBNext Bead Binding Buffer

#E6034A: 2.25 ml **Concentration: 2X**
#E6034AA: 11.25 ml

Store at 4°C

1X NEBNext Bead Binding Buffer:
5 mM Tris-HCl
0.5 mM EDTA
1 M NaCl
pH 7.5 @ 25°C

Description: NEBNext Bead Binding Buffer has been optimized for binding biotin-labeled DNA to Magnetic Streptavidin Beads.

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing this reaction buffer at 0.2X concentration and 1 µg of HaeIII digested ϕX174 RF I 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 0.2X 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 0.2X 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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NEBNext Bead Wash Buffer

#E6033A: 4.0 ml **Concentration: 1X**
#E6033AA: 20.0 ml

Store at 4°C

1X NEBNext Bead Wash Buffer:
5 mM Tris-HCl
0.5 mM EDTA
1 M NaCl
pH 7.5 @ 25°C

Description: NEBNext Bead Wash Buffer has been optimized to remove non-specific binding to Magnetic Streptavidin Beads.

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing this reaction buffer at 0.1X concentration and 1 µg of HaeIII digested ϕX174 RF I 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 0.1X 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 0.1X 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 0.1X 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 0.5X 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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Version 2.1
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