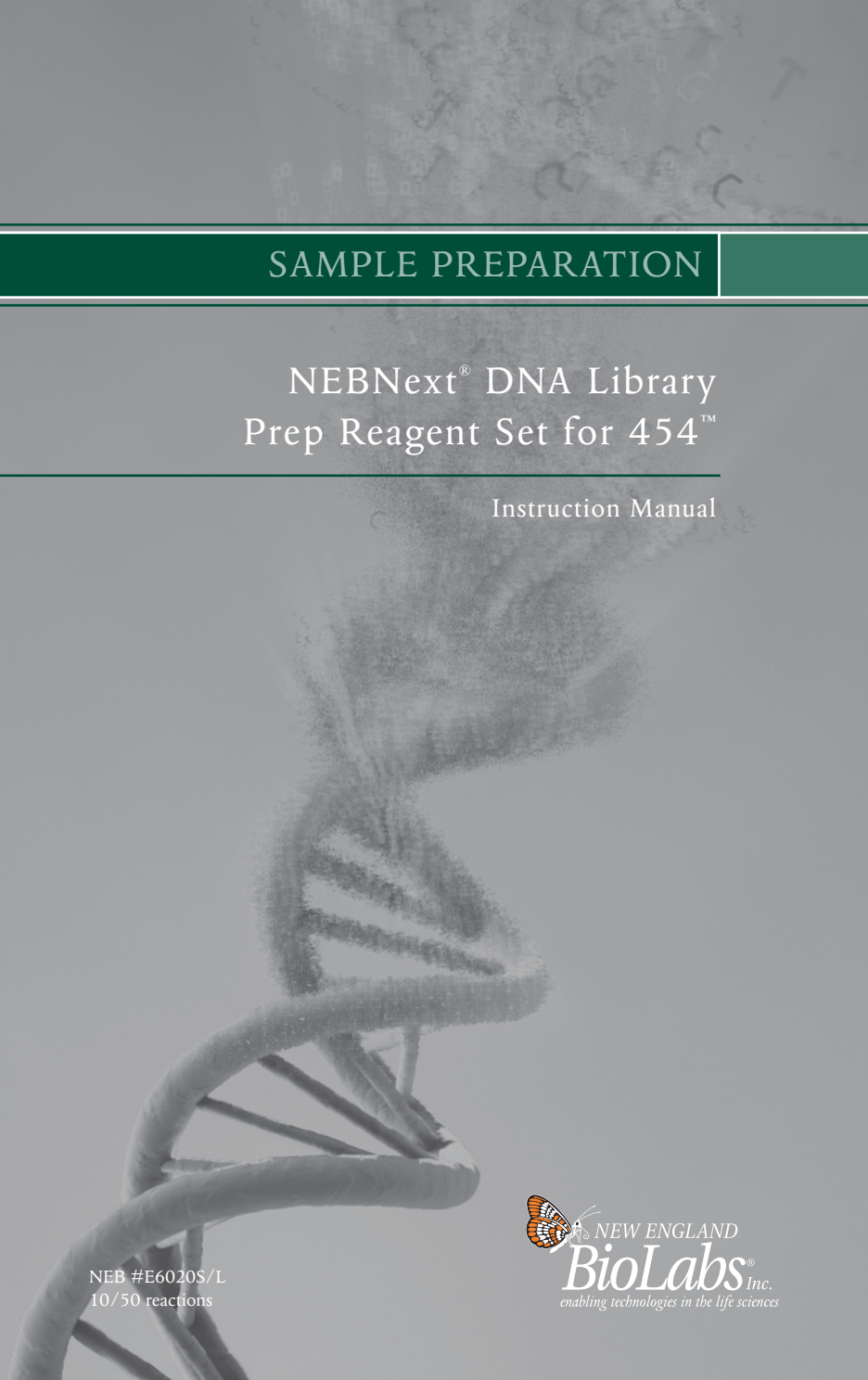
SAMPLE PREPARATION

NEBNext® DNA Library Prep Reagent Set for 454™

Instruction Manual



NEB #E6020S/L
10/50 reactions

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enabling technologies in the life sciences

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Cloned at NEBiolabs


Recombinant Enzyme


Optimum Buffer


Requires BSA


Heat Inactivation

Table of Contents:

The Reagent Set Includes:

The volumes provided are sufficient for preparation of up to 10 reactions (NEB #E6020S) and 50 reactions (NEB #E6020L).

Box 1: Store at –20°C

T4 DNA Polymerase RR

T4 Polynucleotide Kinase RR

Adenosine 5´- Triphosphate (ATP), (10 mM)

Deoxynucleotide Solution Mix (dNTP Mix) (10 mM each)

Bovine Serum Albumin (BSA) (10X)

NEBuffer 2 for T4 DNA Polymerase & T4 Polynucleotide Kinase (10X)

Quick T4 DNA Ligase RR

Quick Ligation Reaction Buffer (2X)

Bst DNA Polymerase, Large Fragment RR

ThermoPol Reaction Buffer for *Bst* DNA Polymerase, Large Fragment (10X)

Molecular Biology Grade Water

Box 2: Store at 4°C

Hydrophilic Streptavidin Magnetic Beads (4 mg/ml)

NEBNext Bead Binding Buffer (2X)

NEBNext Bead Wash Buffer (1X)

Applications

Refer to your specific sample preparation protocol to determine conditions for use.

The NEBNext DNA Library Prep Reagent Set for 454 contains enzymes and buffers 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 Reagent 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.

T4 DNA Polymerase

#E6025A: 0.05 ml
#E6025AA: 0.25 ml

RR BSA Yes

Store at –20°C

Description: T4 DNA Polymerase catalyzes the synthesis of DNA in the 5´→ 3´ direction and requires the presence of template and primer. This enzyme has a 3´→ 5´ exonuclease activity which is much more active than that found in DNA Polymerase I. Unlike *E. coli* DNA Polymerase I, T4 DNA Polymerase does not have a 5´→ 3´ exonuclease function.

Source: Purified from a strain of *E. coli* that carries a T4 DNA Polymerase overproducing plasmid.

Supplied in: 100 mM $KP0_4$ (pH 6.5), 1 mM DTT and 50% glycerol.

Quality Control Assays

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

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 30 units of this enzyme in protein phosphatase assay buffer (1M 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): 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 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.

Lot Controlled

References:

1. Tabor, S. and Struhl, K. (1989). DNA-Dependent DNA Polymerases. In F. M. Ausebel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith and K. Struhl (Eds.), *Current Protocols in Molecular Biology* (pp. 3.5.10–3.5.12). New York: John Wiley & Sons Inc.
2. Sambrook, J. et al. (1989). *Molecular Cloning: A Laboratory Manual,* (2nd ed.), (pp. 5.44–5.47). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

T4 Polynucleotide Kinase

#E6026A: 0.05 ml
#E6026AA: 0.25 ml

Store at –20°C

Description: Catalyzes the transfer and exchange of P_i from the γ position of ATP to the 5´-hydroxyl terminus of polynucleotides (double- and single-stranded DNA and RNA) and nucleoside 3´-monophosphates. Polynucleotide Kinase also catalyzes the removal of 3´-phosphoryl groups from 3´-phosphoryl polynucleotides, deoxynucleoside 3´-monophosphates and deoxynucleoside 3´-diphosphates (1).

Source: An *E. coli* strain that carries the cloned T4 Polynucleotide Kinase gene. T4 Polynucleotide Kinase is purified by a modification of the method of Richardson (1).

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 0.1 µM ATP and 50% glycerol.

Quality Assurance: Free of exonuclease, phosphatase, endonuclease and RNase activities. Each lot is tested under 5´-end-labeling conditions to assure maximal transfer of [^{32}P].

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 10 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 10 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 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 100 units of this enzyme in protein phosphatase assay buffer (1M 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 100 units of this enzyme with 2 µg MS2 phage RNA for 1 hour at 37°C in 50 µl 1X T4 Polynucleotide Kinase Reaction Buffer followed by agarose gel electrophoresis shows no degradation. Incubation of 10 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 300 units of enzyme with 1 µg sonicated [^{3}H] DNA (10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Functional Activity (Labeling): ^{32}P end labeling of 5´-hydroxyl terminated $d(T)_8$ with a minimum of 50 units of this enzyme for 30 minutes at 37°C in 50 µl 1X T4 Polynucleotide Kinase Buffer followed by 20% acrylamide gel electrophoresis reveals that less than 1% of the product has been degraded by exonuclease or phosphatase activities.

Lot Controlled

References:

1. Richardson, C.C. (1981). In P.D. Boyer (Ed.), *The Enzymes* Vol. 14, (pp. 299–314). San Diego: Academic Press.
2. Sambrook, J. et al. (1989) *Molecular Cloning: A Laboratory Manual*, (2nd ed.), (pp. 10.59–10.67, 11.31–11.33). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Adenosine 5´- Triphosphate (ATP)

#E6024A: 0.05 ml **Concentration: 10 mM**
#E6024AA: 0.25 ml

Store at –20°C

Description: Adenosine 5´-Triphosphate (ATP) is a substrate for ATP-dependent enzyme systems.

Supplied in: Sterile purified water adjusted to pH 7.0 with NaOH.

Molecular Weight: 551.2 daltons (disodium salt)

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing a minimum of 0.2 mM ATP 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 0.2 mM ATP 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 a minimum of 0.1 mM ATP 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 1 mM ATP 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

Deoxynucleotide Solution Mix

#E6023A: 0.04 ml **10 mM each dNTP**
#E6023AA: 0.20 ml

Store at –20°C

Description: Deoxynucleotide Solution Mix (dNTP Mix) is an equimolar solution of ultrapure dATP, dCTP, dGTP and dTTP.

Supplied in: Milli-Q® water (Millipore Corporation) as a sodium salt at pH 7.5.

Concentration: Each nucleotide is supplied at a concentration of 10 mM. (40 mM total nucleotide concentration).

Quality Assurance: Nucleotide solutions are certified free of nucleases and phosphatases.

Notes: Storing nucleotide triphosphates in solutions containing magnesium promotes triphosphate degradation.

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing a minimum of 2 mM dNTPs 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 mM dNTPs 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 1 mM dNTPs 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 5 mM dNTPs in protein phosphatase assay buffer (1M 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.

HPLC: dNTP purity is determined by HPLC to be > 99%.

Functional Activity (PCR): The dNTPs are tested in 25 cycles of PCR amplification generating 0.5 kb, 2 kb, and 5kb amplicons from lambda DNA.

Lot Controlled

Bovine Serum Albumin (BSA)

#E6022A: 0.05 ml **Concentration: 10X**
#E6022AA: 0.25 ml

Store at –20°C

Description: Bovine Serum Albumin (BSA) is supplied to prevent adhesion of the enzyme to reaction tubes, pipette surfaces and to stabilize proteins during incubation.

Supplied in: 20 mM KPO_4, 50 mM NaCl, 0.1 mM EDTA, 5% glycerol (pH 7.0 @ 25°C).

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing a minimum of 50 µg of BSA with 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 50 µg of BSA with 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 µg of BSA 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 50 µg of BSA with 1 µg of ssRNA Ladder (NEB #N0362) for 1 hour at 37°C in 50 µl assay buffer results in no detectable RNase activity as determined by polyacrylamide gel electrophoresis.

Incubation of 10 µg of BSA with 40 ng of FAM-Labeled RNA transcript for 16 hours at 37°C in 50 µl assay buffer results in no detectable RNase activity as determined by polyacrylamide gel electrophoresis.

Lot Controlled

NEBuffer 2 for T4 DNA Polymerase & T4 Polynucleotide Kinase

#E6021A: 0.05 ml **Concentration: 10X**
#E6021AA: 0.25 ml

Store at –20°C

1X NEBuffer 2:
50 mM NaCl
10 mM Tris-HCl
10 mM $MgCl_2$
1 mM DTT
pH 7.9 @ 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

#E6028A: 0.05 ml
#E6028AA: 0.25 ml

Store at –20°C

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.

Quick Ligation Reaction Buffer

#E6027A: 0.2 ml **Concentration: 2X**
#E6027AA: 1.0 ml

Store at –20°C

1X Quick Ligation Reaction Buffer:
66 mM Tris-HCl
10 mM $MgCl_2$
1 mM dithiothreitol
1 mM ATP
7.5% 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.

Lot Controlled

Bst DNA Polymerase, Large Fragment

#E6030A: **0.03 ml**
#E6030AA: **0.15 ml**

Store at –20°C

1X ThermoPol Reaction Buffer:
20 mM Tris-HCl
10 mM KCl
10 mM $(NH_4)_2SO_4$
2 mM $MgSO_4$
0.1% Triton X-100
pH 8.8 @ 25°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.

ThermoPol Reaction Buffer for *Bst* DNA Polymerase, Large Fragment

#E6029A: 0.05 ml **Concentration: 10X**
#E6029AA: 0.25 ml

Store at –20°C

1X ThermoPol 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
pH 8.8 @ 25°C

Description: New England Biolabs supplies a unique 10X reaction buffer for *Bst* DNA Polymerase, Large Fragment

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: 2 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 (50 mM 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 (50 mM 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 (50 mM 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.

Lot Controlled

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.

Lot Controlled

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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USA
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1/12