

SAMPLE PREPARATION

NEBNext[®] Small RNA Library Prep Set for Illumina[®]

Instruction Manual

NEB #E6120S/L
10/50 reactions

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



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The Set Includes:

The volumes provided are sufficient for preparation of up to 10 reactions (NEB #E6120S) and 50 reactions (NEB #E6120L). (All reagents should be stored at -20°C).

NEBNext 3' Ligation Reaction Buffer (2X)
 NEBNext 3' Ligation Enzyme Mix
 NEBNext 3' SR Adaptor 1
 NEBNext 5' SR Adaptor 1
 NEBNext 5' Ligation Reaction Buffer (10X)
 NEBNext 5' Ligation Enzyme Mix
 NEBNext SR RT Primer 1
 Deoxynucleotide Solution Mix (10 mM each dNTP)
 Murine RNase Inhibitor
 LongAmp Taq 2X Master Mix
 NEBNext SR Primer F1
 NEBNext SR Primer R1
 Gel Loading Dye, Blue (6X)
 Quick-Load Low Molecular Weight DNA Ladder
 DNA Gel Elution Buffer, 1X
 Linear Acrylamide (10 mg/ml)
 TE Buffer
 Nuclease-free Water

Required Materials Not Included:

3 M Sodium Acetate, pH 5.2
 100% Ethanol
 80% Ethanol
 SuperScript[®] II Reverse Transcriptase (Life Technologies, Inc. #18064-014)
 5X First Strand Buffer (Supplied with SuperScript II Reverse Transcriptase)
 0.1 M DTT (Supplied with SuperScript II Reverse Transcriptase)
 Corning[®], Costar[®], Spin-X[®] Centrifuge Tube Filters (Cellulose Acetate Filters) (Sigma Aldrich # CLS8162)
 RNase-free Disposable Pellet Pestles[®] (Kimble Kontes Asset Management, Inc. #749521-1590)
 6% Novex[®] TBE PAGE gel, 1.0 mm, 10 well (Life Technologies, Inc. #EC6265BOX)
 SYBR[®] Gold Nucleic Acid Gel Stain (Life Technologies, Inc. S-11494)

Applications:

The NEBNext Small RNA Library Prep Set for Illumina contains adaptors, primers, enzymes and buffers that are ideal to convert small RNA into cDNA libraries for next-generation sequencing on the Illumina platform (Illumina, Inc.). 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 Small RNA Library Prep Set for Illumina 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 small RNA library on the Illumina sequencing platform.

For larger volume requirements, customized and bulk packaging is available by purchasing through the OEM/Bulks department at NEB. Please contact OEM@neb.com for further information.

Small RNA Library Prep Workflow:

Novel Protocol that Results in Higher Yields and Lower Adaptor-dimer Contamination (Patent Pending):

Workflow for Small RNA Library Prep	Reagents Supplied	Required Materials, Not Supplied
3' Ligation 	<ul style="list-style-type: none"> 3' Ligation Enzyme Mix 3' Ligation Reaction Buffer (2X) 3' SR Adaptor 3 	<ul style="list-style-type: none"> Input RNA (Total RNA, or previously enriched small RNA)
Primer Hybridization* 	<ul style="list-style-type: none"> SR RT Primer 3 	
5' Ligation 	<ul style="list-style-type: none"> 5' Ligation Enzyme Mix 5' Ligation Reaction Buffer (10X) 5' SR Adaptor 3 Nuclease-Free Water 	
First Strand Synthesis 	<ul style="list-style-type: none"> Murine RNase Inhibitor dNTPs 	<ul style="list-style-type: none"> SuperScript II Reverse Transcriptase First Strand Buffer DTT
PCR Amplification 	<ul style="list-style-type: none"> LongAmp Taq 2X Master Mix SR Primer F3 SR Primer R3 	
Size Selection 	<ul style="list-style-type: none"> Gel Loading Dye, Orange (6X) Quick-Load Low Molecular Weight DNA Ladder DNA Gel Elution Buffer (1X) Linear Acrylamide TE 	<ul style="list-style-type: none"> 6% PAGE Gel SYBR Gold Nucleic Acid Gel Stain RNase-free Disposable Pellet Pestles Filtration Spin Column 100% Ethanol 80% Ethanol 3M Sodium Acetate, pH 5.2

KEY: RNA = Red
 Adaptor = Green
 RT Primer = Aqua
 cDNA = Blue
 DNA Product = Black

*This step is important to prevent adaptor-dimer formation. The RT primer hybridizes to the excess of 3' adaptor (that remains unligated after the 3' ligation reaction) and transforms the single strand DNA adaptor into a double strand DNA molecule. dsDNA are not substrate for ligations mediated by T4 RNA ligase 1 and therefore do not ligate to the 5' adaptor in the subsequent ligation step.

Protocols:

Starting Material: 1–10 µg total RNA. Alternatively, previously isolated small RNA from 1–10 µg total RNA can be used as starting material.

Ligation of 3' and 5' Adaptors

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

Input RNA	1–6 µl
3' SR Adaptor 1	1 µl
Nuclease-free Water	variable
Total volume	7 µl

2. Incubate in a preheated thermal cycler for 2 minutes at 70°C.

3. Transfer tube to ice.

4. Add the following components:

3' Ligation Reaction Buffer (2X)	10 µl
3' Ligation Enzyme Mix	3 µl
Total volume	20 µl

5. Incubate for 1 hour at 25°C in a thermal cycler.

6. Add the following components to the ligation mixture from step 5 and mix well:

Nuclease-free Water	4.5 µl
SR RT Primer 1	1 µl
Total volume now should be	25.5 µl

7. Heat samples for 5 minutes at 75°C. Transfer to 37°C for 30 minutes and then, to 25°C for 15 minutes.

8. With 5 minutes remaining, resuspend the 5' SR Adaptor 1 in Nuclease-free Water (For NEB #E6120S resuspend NEB #E6125A in 60 µl Nuclease-free Water and for NEB #E6120L resuspend NEB #E6125AA in 300 µl Nuclease-free Water).

9. Heat the adaptor at 70°C for 2 minutes.

10. Transfer tube to ice.

11. Add the following components to the ligation mixture from step 7 and mix well:

5' SR Adaptor 1 (from Step 10)	1 µl
5' Ligation Reaction Buffer (10X)	1 µl
5' Ligation Enzyme Mix	2.5 µl
Total volume now should be	30 µl

12. Incubate for 1 hour at 25°C in a thermal cycler.

Reverse Transcription

1. Mix the following components in a sterile nuclease-free tube:

3' 5' Ligated RNA from step 12	11 µl
Deoxynucleotide Solution Mix	1 µl
5X First Strand Buffer (Supplied with SuperScript II)	4 µl
0.1 M DTT (Supplied with SuperScript II)	2 µl
Murine RNase Inhibitor	1 µl
Total volume	19 µl

2. Heat the sample for 2 minutes at 42°C.

3. Add 1 µl SuperScript II Reverse Transcriptase.

4. Incubate for 1 hour at 42°C.

PCR Amplification

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

RT reaction mixture	20 µl
LongAmp <i>Taq</i> 2X Master Mix	25 µl
SR Primer F1	2.5 µl
SR Primer R1	2.5 µl
Total volume	50 µl

2. PCR cycling conditions:

CYCLE STEP	TEMP	TIME	CYCLES
Initial Denaturation	94°C	30 sec	1
Denaturation	94°C	10 sec	12*
Annealing	60°C	30 sec	
Extension	65°C	15 sec	
Final Extension	65°C	5 min	1
Hold	4°C		

*Amplification conditions may vary based on RNA input amount, tissue, and species. This protocol was optimized using 5 µg of total RNA from human brain. The number of PCR cycles can be adjusted if clear and distinct bands are not observed in the gel image. However, only run between 12 and 15 cycles.

Size Selection and Gel Purification of Amplified cDNA Library

1. Mix 50 μ l of amplified cDNA construct with 10 μ l of Gel loading dye, Blue (6X).
2. Load 10 μ l of Quick-Load Low Molecular Weight DNA Ladder in one well on the 6% PAGE gel.
3. Load two wells with 30 μ l each of mixed amplified cDNA construct and loading dye on the 6% PAGE gel.
4. Run the gel for 60 minutes at 120 V or until the front of the dye reach the bottom of the gel. Do not let the dye exit the gel.
5. Remove the gel from the apparatus and stain the gel with SYBR Gold nucleic acid gel stain in a clean container for 2–3 minutes and view the gel on a UV transilluminator.
6. Cut the bands corresponding to ~90–100 bp. The 91 and 100 nucleotide bands correspond to adaptor-ligated constructs derived from the 21 and 30 nucleotide RNA fragments, respectively. Do not cut the 70 bp band out, as this is adaptor dimer. (Figure 1)

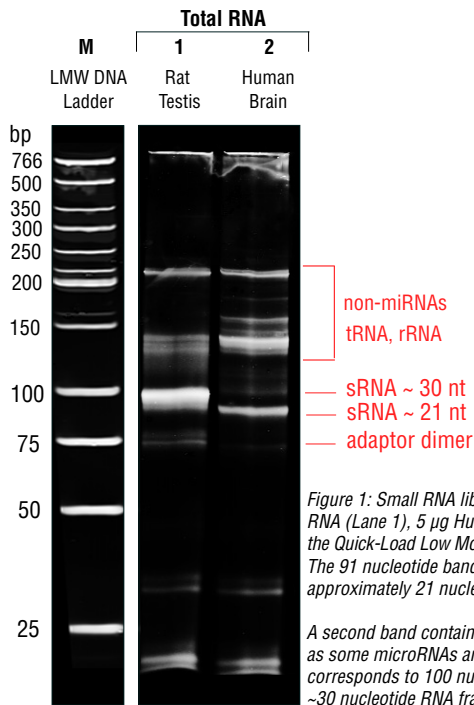


Figure 1: Small RNA libraries generated from 5 μ g Rat Testis Total RNA (Lane 1), 5 μ g Human Brain Total RNA (Lane 2) and 10 μ l of the Quick-Load Low Molecular Weight Ladder (M), NEB #N0474. The 91 nucleotide band contains mature miRNA generated from approximately 21 nucleotide small RNA fragments.

A second band containing piwi-interacting RNAs (piRNAs) as well as some microRNAs and other regulatory small RNA molecules, corresponds to 100 nucleotides in length and is generated from ~30 nucleotide RNA fragments.

7. Place the gel slice in a 1.5 ml tube and crush the gel slice with the RNase-free Disposable Pellet Pestles and soak in 250 μ l DNA Gel Elution Buffer (1X).
8. Rotate for 2–18 hours at room temperature.
9. Transfer the eluate and the gel debris to the top of a gel filtration column.
10. Centrifuge the filter for 2 minutes at 14,000 rpm.
11. Recover eluate and add 1 μ l Linear Acrylamide, 25 μ l 3M sodium acetate pH 5.2 and 750 μ l of 100% ethanol.
12. Vortex well.
13. Precipitate in a dry ice/methanol bath for at least 30 minutes.
14. Spin in a microcentrifuge (>14,000 x g) for 30 minutes at 4°C.
15. Remove the supernatant taking care not to disturb/remove the pellet.
16. Wash the pellet with 80% ethanol by vortexing vigorously.
17. Spin in a microcentrifuge (>14,000 x g) for 30 minutes at 4°C.
18. Air dry pellet for up to 10 minutes at room temperature to remove residual ethanol.
19. Resuspend pellet in 10 μ l TE Buffer. Perform the following quality control analysis on your sample library to quantify the DNA concentration.
20. Load 1 μ l of the resuspended construct on an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.) using a DNA specific chip such as the Agilent DNA-1000 (Figure 2).

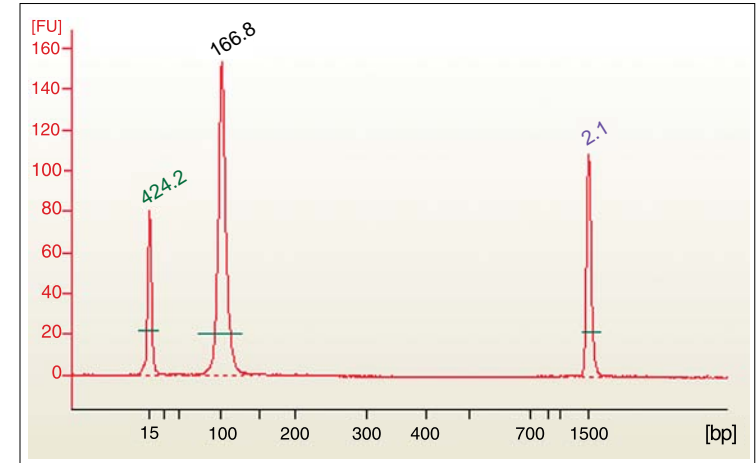


Figure 2: Agilent Bioanalyzer Trace of a Final Human Brain miRNA Library showing a 166.8 nM single peak.

21. Check the size, purity and concentration of the sample. The final product should be a distinct band. If you see undesirable peaks (bigger or smaller than your expected range sizes) perform a second round of size selection.

NEBNext 3' Ligation Reaction Buffer

#E6121A: 0.1 ml

Concentration: 2X

#E6121AA: 0.5 ml

Store at -20°C

1X NEBNext 3' Ligation Reaction Buffer:

50 mM Tris-HCl

10 mM MgCl₂

1 mM DTT

12.5% Polyethylene glycol (PEG 8,000)

pH 7.5 @ 25°C

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1X 3' Ligation 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 3' Ligation 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 50 µl reaction containing 1X 3' Ligation Reaction Buffer 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.

Phosphatase Activity: Incubation of 1X 3' Ligation Reaction Buffer 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 a 10 µl reaction containing 1X 3' Ligation Reaction Buffer with 40 ng of a FAM-labeled RNA transcript for 16 hours at 37°C resulted in no detectable degradation of RNA as determined by polyacrylamide gel electrophoresis.

Lot Controlled

NEBNext 3' Ligation Enzyme Mix

#E6122A: 0.03 ml

#E6122AA: 0.15 ml

Store at -20°C

Description: NEBNext 3' Ligation Enzyme Mix has been optimized to ligate short single-stranded RNAs (17–30 nucleotide length) to a 5'-adenylated, 3'-blocked single-stranded DNA adaptor in 1X NEBNext 3' Ligation Reaction Buffer at 25°C.

NEBNext 3' Ligation Enzyme Mix:

133,333 units/ml T4 RNA Ligase 2, truncated,

13,333 units/ml Murine RNase Inhibitor

Supplied in: 10 mM Tris-HCl (pH 7.5 @ 25°C), 100 mM NaCl, 0.1 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.

16-Hour Incubation: 50 µl reactions containing 1 µl of 3' Ligation Reaction Enzyme Mix and 1 µg of HindIII digested Lambda 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 of 3' Ligation Reaction Enzyme Mix 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 3' Ligation Reaction Enzyme Mix 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 3' Ligation Reaction Enzyme Mix with 40 ng of a FAM-labeled RNA transcript for 16 hours at 37°C resulted in no detectable degradation of RNA as determined by polyacrylamide gel electrophoresis.

Phosphatase Activity: Incubation of a minimum of 1 µl 3' Ligation Reaction Enzyme Mix 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.

Functional Activity: 200 units of T4 RNA Ligase 2, truncated, ligates 80% of a 31-mer RNA to the pre-adenylated end of a 17-mer DNA [Universal miRNA Cloning Linker (NEB #S1315)] in a total reaction volume of 10 µl in 1 hour at 25°C. Unit Assay Conditions: 1X Reaction Buffer (50 mM Tris-HCl, 10 mM MgCl₂, 1 mM DTT, pH 7.5 @ 25°C) supplemented to 10% (w/v) PEG MW 4000, 5 pmol of 5'-FAM labeled RNA, and 10 pmol preadenylated DNA linker. After incubation at 25°C for 1 hour, the ligated product is detected on a 15% denaturing polyacrylamide gel.

One unit of Murine RNase Inhibitor inhibits the activity of 5 ng of RNase A by 50%. Activity is measured by the inhibition of hydrolysis of cytidine 2, 3'-cyclic monophosphate by RNase A.

Lot Controlled

NEBNext 3' SR Adaptor 1

#E6124A: 15 µl

#E6124AA: 50 µl

Store at -20°C

Description: 5' adenylated, 3' blocked oligodeoxynucleotide

Sequence: rAppTCGTATGCCGTCTTCTGCTTG-NH₂-3'

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1 µl NEBNext 3' SR Adaptor 1 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 NEBNext 3' SR Adaptor 1 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 3' SR Adaptor 1 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 3' SR Adaptor 1 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 NEBNext 3' SR Adaptor 1 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.

HPLC: NEBNext 3' SR Adaptor 1 purity is determined by HPLC to be > 99%.

Lot Controlled

NEBNext 5' SR Adaptor 1

#E6125A: 1,350 pmol

#E6125AA: 6,750 pmol

Store at -20°C

Description: Single-Stranded oligoribonucleotide (26 nucleotide length)

Sequence: 5' - rGrUrUrCrArGrArGrUrUrCrUrArCrArGrUrCrCrGrArCrGrArUrC-3'

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1 µl NEBNext 5' SR Adaptor 1 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 NEBNext 5' SR Adaptor 1 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 5' SR Adaptor 1 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 5' SR Adaptor 1 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 NEBNext 5' SR Adaptor 1 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.

HPLC: NEBNext 5' SR Adaptor 1 purity is determined by HPLC to be > 99%.

Lot Controlled

NEBNext 5' Ligation Reaction Buffer

#E6126A: 15 µl
#E6126AA: 50 µl

Concentration: 10X

Store at -20°C

1X NEBNext 5' Ligation Reaction Buffer:

50 mM Tris-HCl
10 mM MgCl₂
1 mM DTT
3 mM ATP
pH 7.5 @ 25°C

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1X 5' Ligation 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 5' Ligation 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 50 µl reaction containing 1X 5' Ligation Reaction Buffer 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 1X 5' Ligation Reaction Buffer with 40 ng of a FAM-labeled RNA transcript for 16 hours at 37°C resulted in no detectable degradation of RNA as determined by polyacrylamide gel electrophoresis.

Phosphatase Activity: Incubation of 1X 5' Ligation Reaction Buffer 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 5' Ligation Enzyme Mix

#E6127A: 0.025 ml
#E6127AA: 0.125 ml

Store at -20°C

Description: NEBNext 5' Ligation Enzyme Mix has been optimized to ligate short single-stranded RNAs (17–30 nucleotide length) in 1X NEBNext 5' Ligation Reaction Buffer at 25°C.

NEBNext 5' Ligation Enzyme Mix:

2,568 units/ml T4 RNA Ligase 1
16,000 units/ml Murine RNase Inhibitor

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.

16-Hour Incubation: 50 µl reactions containing 1 µl of 5' Ligation Reaction Enzyme Mix 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 of 5' Ligation Reaction Enzyme Mix 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 5' Ligation Reaction Enzyme Mix 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.

Phosphatase Activity: Incubation of a minimum of 1 µl 5' Ligation Reaction Enzyme Mix 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.

Functional Activity: One unit of T4 RNA Ligase 1 is defined as the amount of enzyme required to convert 1 nanomole of 5'-[³²P]rA₁₆ into a phosphatase-resistant form in 30 minutes at 37°C.

One unit of Murine Inhibitor inhibits the activity of 5 ng of RNaseA by 50%. Activity is measured by the inhibition of hydrolysis of cytidine 2, 3'-cyclic monophosphate by RNaseA

Lot Controlled

NEBNext SR RT Primer 1

#E6128A: 15 µl

#E6128AA: 50 µl

Store at -20°C

Description: Single-stranded oligodeoxynucleotide (21 nucleotide length)

Sequence: 5'-CAAGCAGAAGACGGCATAACGA-3'

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1 µl NEBNext SR RT Primer 1 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 SR RT Primer 1 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 SR RT Primer 1 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 SR RT Primer 1 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 NEBNext SR RT Primer 1 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

Deoxynucleotide Solution Mix

#E6202A: 0.02 ml

10 mM each dNTP

#E6202AA: 0.1 ml

Store at -20°C

Description: Deoxynucleotide Solution 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 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 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 (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.

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

Murine RNase Inhibitor

#E6123A: 15 µl
#E6123AA: 50 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 [³H] *E. coli* DNA (20⁵ 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 300 ng supercoiled plasmid for 4 hours at 37°C produced no 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 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

References:

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

LongAmp *Taq* 2X Master Mix



E6129A: 0.25 ml Concentration: 2X
E6129AA: 1.25 ml

Store at -20°C

1X LongAmp *Taq* Master Mix:

60 mM Tris-SO₄
2 mM MgSO₄
0.3 mM dNTPs
125 units/ml LongAmp *Taq* DNA Polymerase
20 mM ammonium sulfate
pH 9.0 @ 25°C

Quality Control Assays

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

16-Hour Incubation: 50 µl reactions containing a minimum of 5 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 5 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.

RNase Activity: Incubation of a 10 µl reaction containing 1 µl of LongAmp *Taq* 2X Master Mix with 40 ng of a FAM-labeled RNA transcript for 16 hours at 37°C resulted in no detectable degradation of RNA as determined by polyacrylamide gel electrophoresis.

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 (nicked molecules) 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 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.

Functional Activity: One unit of LongAmp *Taq* DNA Polymerase is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble material in 1X ThermoPol Reaction Buffer, 200 µM dNTPs including [³H]-dTTP and 200 µg/ml activated Calf Thymus DNA; in 30 minutes at 65°C.

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Purchase of this product provides the purchaser with a non-exclusive license to use LongAmp® *Taq* 2X Master Mix for research purposes only. Commercial use of this product may require a license from New England Biolabs, Inc. under U.S. Patent No. 5,352,778.

The purpose of this product also conveys to the purchaser only the limited, non-transferable right to use the purchased quantity of the product for the purchaser's own research by the purchaser only under the following U.S. patent claims and foreign counterpart patent claims: U.S. Patent No. 5,436,149 (claims 6-16). No rights are granted to the purchaser to sell, modify for resale or otherwise transfer this product, either alone or as a component of another product, to any third party. Takara Bio reserves all other rights, and this product may not be used in any manner other than as provided herein. For information on obtaining a license to use this product for purposes other than research, please contact Takara Bio Inc., Seto 3-4-1, Otsu, Shiga 520-2193 Japan (Fax: +81-77-453-9254).

NEBNext SR Primer F1

#E6130A: 0.025 ml

#E6130AA: 0.125 ml

Store at -20°C

Sequence: 5'-CAAGCAGAAGACGGCATACG-s-A-3'

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1 µl NEBNext SR Primer F1 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 SR Primer F1 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.

RNase Activity: Incubation of a 10 µl reaction containing 1 µl NEBNext SR Primer F1 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 50 µl reaction containing 1 µl NEBNext SR Primer F1 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.

Phosphatase Activity: Incubation of 1 µl NEBNext SR Primer F1 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.

HPLC: NEBNext SR Primer F1 purity is determined by HPLC to be > 99%.

Lot Controlled

NEBNext SR Primer R1

#E6131A: 0.025 ml

#E6131AA: 0.125 ml

Store at -20°C

Sequence: 5'-AATGATACGGCGACCACCGACAGGTTTCAGAGTTCTACAGTCCG-s-A-3'

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1 µl NEBNext SR Primer R1 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 SR Primer R1 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.

RNase Activity: Incubation of a 10 µl reaction containing 1 µl NEBNext SR Primer R1 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 50 µl reaction containing 1 µl NEBNext SR Primer R1 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.

Phosphatase Activity: Incubation of 1 µl NEBNext SR Primer R1 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

Gel Loading Dye, Blue

#E6138A: 0.2 ml

Concentration: 6X

#E6138AA: 1 ml

Store at 25°C

Description: Gel Loading Dye, Blue (6X) is a pre-mixed loading buffer with a tracking dye for agarose and non-denaturing polyacrylamide gel electrophoresis. This solution contains SDS, which often results in sharper bands, as some enzymes are known to remain bound to their DNA substrates following cleavage. EDTA is also included to chelate magnesium (up to 10 mM) in enzymatic reactions, thereby stopping the reaction. Bromophenol Blue migrates at approximately 300 bp on a standard 1% TBE agarose gel.

1X Gel Loading Dye, Blue (6X):

2.5% Ficoll 400

11 mM EDTA

3.3 mM Tris-HCl

0.017% SDS

0.15% Bromophenol Blue

pH 8.0 @ 25°C

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1X Gel Loading Dye 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 Gel Loading Dye 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 1X Gel Loading Dye 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 1X Gel Loading Dye 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 Gel Loading Dye 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

Quick-Load Low Molecular Weight DNA Ladder

#E6133A: 0.1 ml

Concentration: 50 µg/ml

#E6133AA: 0.5 ml

Store at 4°C

Description: Quick-Load Low Molecular Weight DNA Ladder is a pre-mixed, ready-to-load molecular weight marker containing bromophenol blue as a tracking dye.

The DNA Ladder consists of a proprietary plasmid which is digested to completion with appropriate restriction enzymes to yield 11 bands suitable for use as molecular weight standards for agarose gel electrophoresis. This digested DNA includes fragments ranging from 25–766 base pairs. The 200 base pair band has increased intensity to serve as a reference point.

Note: To provide increased intensity for the 200 bp reference band, multiple fragments of the same size have been cloned into the plasmid used for this DNA ladder. These fragments, identical in size, are indistinguishable on agarose gels, but, on acrylamide gels, even slight differences in DNA sequence can lead to noticeably different migration rates. Fragments of the same size do not always run the same on acrylamide. This results in two or more of the bands comprising the 200 bp reference band running differently, resulting in one or more extra bands detectable around the 200 bp range.

Storage Conditions:

3.3 mM Tris-HCl

11 mM EDTA

0.015% Bromophenol Blue

0.017% SDS

2.5% Ficoll 400

pH 8.0 @ 25°C

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1 µl Quick-Load Low Molecular Weight Ladder 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 Quick-Load Low Molecular Weight Ladder 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 Quick-Load Low Molecular Weight Ladder 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 Quick-Load Low Molecular Weight Ladder 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 Quick-Load Low Molecular Weight Ladder 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

DNA Gel Elution Buffer

#E6134A: 5 ml
#E6134AA: 25 ml

Concentration: 1X

Store at 4°C

Description: DNA Gel Elution Buffer is provided for the extraction of the size selected amplified cDNA library from the polyacrylamide gel.

DNA Gel Elution Buffer:

10 mM Tris-HCl
pH 8.0 @ 25°C

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 10 µl DNA Gel Elution 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 10 µl DNA Gel Elution 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 50 µl reaction containing 10 µl DNA Gel Elution Buffer 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 DNA Gel Elution 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 DNA Gel Elution Buffer 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

Linear Acrylamide

#E6103A: 20 µl
#E6103AA: 100 µl

Concentration: 10 mg/ml

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 50 µl reaction containing 1 µg Linear Acrylamide 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 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 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

TE Buffer

#E6135A: 0.3 ml

#E6135AA: 1.5 ml

Store at -20°C or 4°C

TE Buffer:

10 mM Tris-HCl

1 mM EDTA pH 8.0

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 10 µl TE 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 10 µl TE 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 50 µl reaction containing 10 µl TE Buffer 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 TE 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 TE Buffer 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

Nuclease-free Water

#E6136A: 4 ml

#E6136AA: 8 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 produced no 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 Nuclease-free Water 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.

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