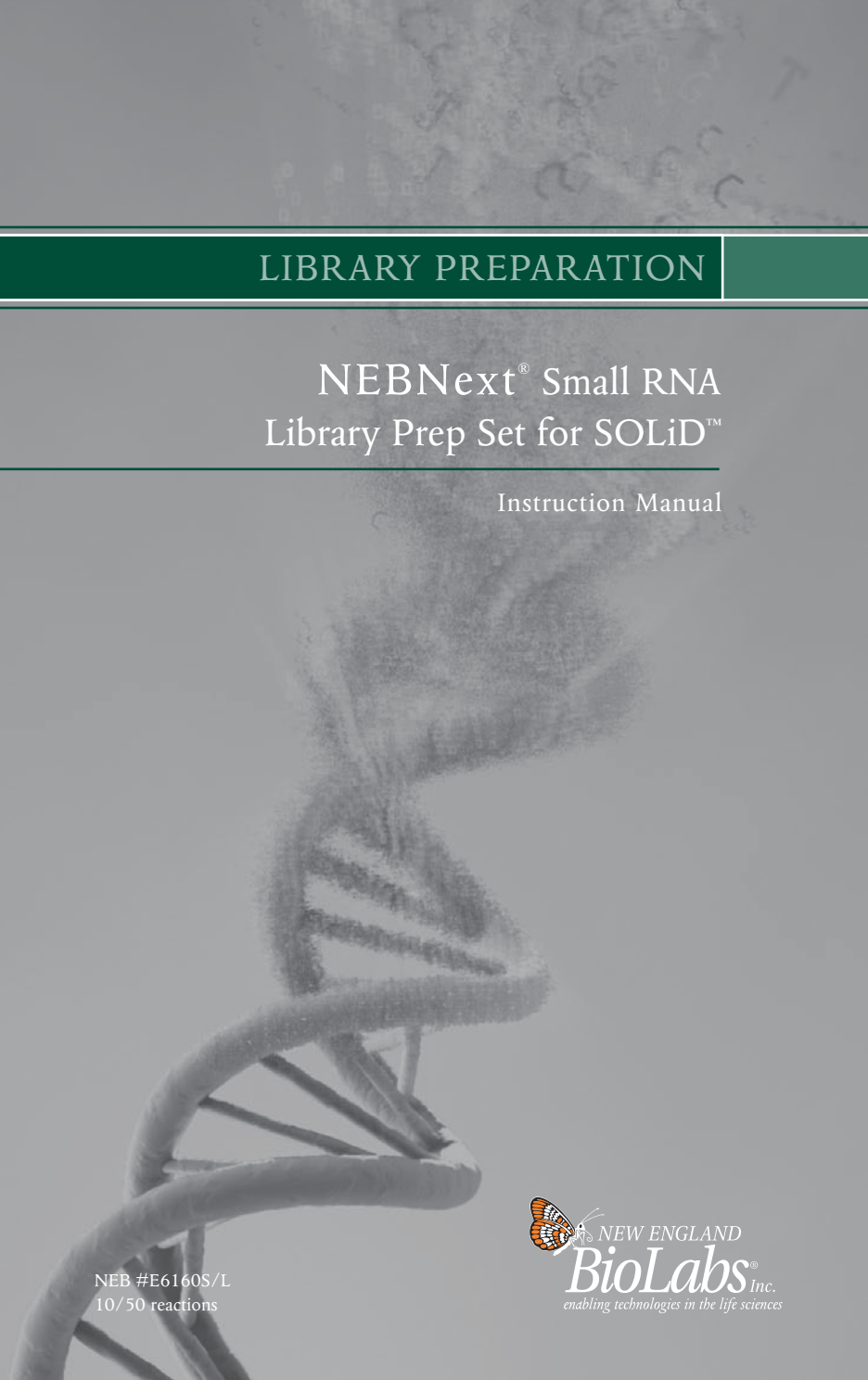
LIBRARY PREPARATION

NEBNext® Small RNA Library Prep Set for SOLiD™

Instruction Manual



NEB #E6160S/L
10/50 reactions

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

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Table of Contents:

The NEBNext Small RNA Library Prep Set for SOLiD Includes:

The volumes provided are sufficient for preparation of up to 10 reactions (NEB #E6160S) and 50 reactions (NEB #E6160L). All reagents should be stored at –20°C. Colored bullets represent the color of the cap of the tube containing the reagent.

- (green) NEBNext 3´ Ligation Reaction Buffer (2X)
- (green) NEBNext 3´ Ligation Enzyme Mix
- (green) NEBNext 3´ SR Adaptor 3
- (yellow) NEBNext 5´ SR Adaptor 3
- (yellow) NEBNext 5´ Ligation Reaction Buffer (10X)
- (yellow) NEBNext 5´ Ligation Enzyme Mix
- (pink) NEBNext SR RT Primer 3
- (red) NEBNext First Strand Synthesis Reaction Buffer
- (red) M-MuLV Reverse Transcriptase (RNase H^-)
- (red) Murine RNase Inhibitor
- (blue) One*Taq* Hot Start 2X Master Mix with Standard Buffer
- (blue) NEBNext Index 1 Primer for SOLiD
- (blue) NEBNext SR Primer R3
- (orange) Gel Loading Dye, Blue (6X)
- (orange) Quick-Load pBR322 DNA-MspI Digest

DNA Gel Elution Buffer, 1X

- (white) Linear Acrylamide (10 mg/ml)
- (white) TE Buffer

Nuclease-free Water

Required Materials Not Included:

3M Sodium Acetate. pH 5.2

100% Ethanol

80% Ethanol

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., E-C6265BOX)

SYBR® Gold Nucleic Acid Gel Stain (Life Technologies, Inc., S-11494)

Applications:

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

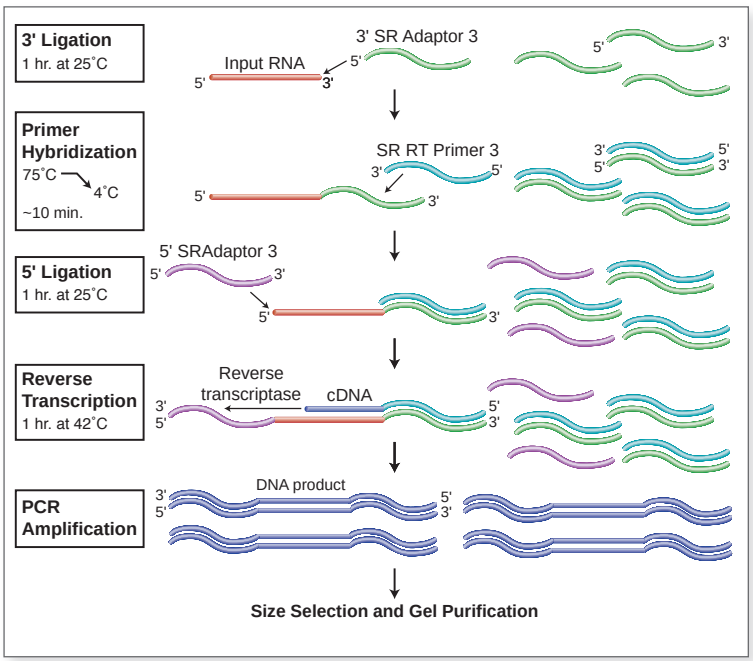
For larger volume requirements, customized and bulk packaging is available by purchasing through the OEM/Bulks department at NEB. Please contact OEM@neb.com for further information.

Kit Optimization:

The components of this kit were collectively optimized with FirstChoice® Human Brain Reference RNA (Life Technologies, Inc. #AM6050).

Small RNA Library Prep Workflow:

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



Protocols:

Symbols

This is a point where you can safely stop the protocol and store the samples at –20°C for up to 72 hours.

This caution sign signifies a step in the protocol that has two paths leading to the same end point but is dependent on a user variable, like the amount of input DNA.

Colored bullets indicate a reagent to be added and the color of it's cap.

Starting Material: 1–10 µg total RNA. Use half of the listed volume for the following reagents if your input is < 5 µg.

(green) 3´ SR Adapter 3

(pink) SR RT Primer 3

(yellow) 5´ SR Adapter 3

It is preferable that all reactions be done in strip tubes and incubated on a thermalcycler.

Ligation of 3´ and 5´ Adaptors (~1 hour and 15 minutes to the first safe stop point)

1. Mix the following components in sterile nuclease-free strip tubes:

Input RNA	1–6 µl
(green) NEBNext 3´ SR Adaptor 3	1 µl
Nuclease-free Water	variable
Total volume	7 µl

2. Incubate in a preheated thermal cycler at 70°C for 2 minutes and transfer tube to ice.

3. Add the following components:

(green) 3´ Ligation Reaction Buffer	10 µl
(green) 3´ Ligation Enzyme Mix	3 µl
Total volume	20 µl

4. Incubate at 25°C for 1 hour in a thermal cycler. The sample can be safely stored at –20°C for 72 hours at this point. ~1 hours and 15 minutes to the next safe stop point.

5. Add the following components to the ligation mixture from step 4 and mix well:

Nuclease-free Water	4.5 µl
(pink) SR RT Primer 3	⚠ 1 µl
Total volume	25.5 µl

6. Heat samples at 75°C for 3 minutes then ramp to 4°C at a rate of 0.3°C/sec and transfer to ice.

7. Resuspend the (yellow) 5´ SR adaptor 3 in Nuclease-free Water (For NEB #E6160S, resuspend in 30 µl of Nuclease-free water and for E6160L resuspend #E6162AA in 150 µl Nuclease-free Water). Heat the resuspended adaptor at 70°C for 2 minutes and transfer to ice (only heat denature once).

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

(yellow) 5´ SR Adaptor 3 (from Step 7)	⚠ 1 µl
(yellow) 5´ Ligation Reaction Buffer	1 µl
(yellow) 5´ Ligation Enzyme Mix	2.5 µl
Total volume	30 µl

9. Incubate at 25°C for 1 hour in a thermal cycler. SAFE STOP The sample can be safely stored at –20°C for 72 hours at this point. ~1 hour and 30 minutes to the next safe stop point.

Reverse Transcription

10. Mix the following components in sterile nuclease-free strip tubes:

3´→5´ Ligated RNA from step 9	14 µl
(red) NEBNext First Strand Synthesis Reaction Buffer	4 µl
(red) Murine RNase Inhibitor	1 µl
Total volume	19 µl

11. Heat the sample at 42°C for 2 minutes and then add the following component.

Preheated RT buffer and sample mix	19 µl
(red) M-MuLV (RNase H⁻) Reverse Transcriptase	1 µl
Total volume	20 µl

12. Incubate mixture for 42°C for 1 hour and then at 70°C for 15 minutes. SAFE STOP The sample can be safely stored at –20°C for 72 hours at this point.

PCR Amplification

13. Mix the following components in sterile strip tubes:

RT reaction mixture (from step 12)	20 µl
(blue) One*Taq* Hot Start Master Mix	25 µl
(blue) Index 1 Primer	2.5 µl
(blue) SR Primer R3	2.5 µl
Total volume	50 µl

14. PCR cycling conditions:

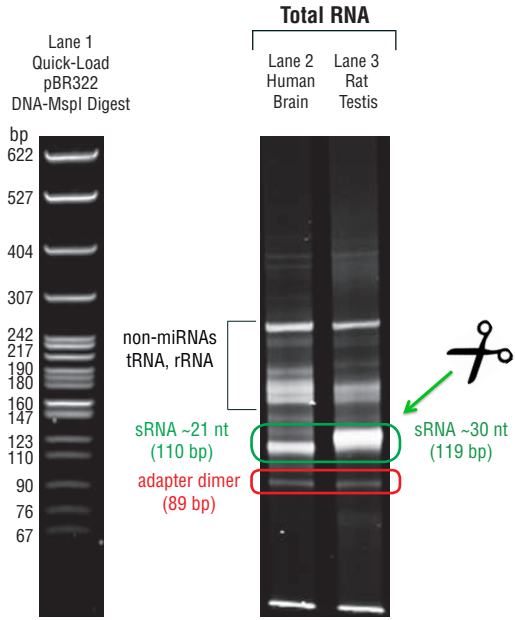
CYCLE STEP	TEMP	TIME	CYCLES
Initial Denaturation	94°C	30 sec	1
Denaturation	94°C	10 sec	
Annealing	60°C	30 sec	12–15
Extension	65°C	15 sec	
Final Extension	65°C	5 min	1
Hold	4°C	∞	

*Amplification conditions may vary based on RNA input amount, tissue and species. However, only run between 12–15 cycles.

Size Selection of Amplified cDNA Library

1. Add 10 µl of (orange) Gel Loading Dye, Blue (6X) to each amplified cDNA construct (60 µl total volume).
2. Load 5 µl of (orange) Quick-Load pBR322 DNA-MspI Digest in one well on a 6% PAGE gel.
3. Load each amplified cDNA construct with loading dye by splitting into two wells (30 µl each) on the 6% PAGE gel.
4. Run the gel at 120 V until the front of the dye reaches the bottom of the gel (~60 minutes). Do not let the dye exit the gel.
5. Remove the gel from the apparatus and stain with SYBR Gold nucleic acid gel stain in a clean container for 10 minutes on orbital shaker and view the gel on a UV transiluminator.
6. Cut the bands corresponding to ~110–119 bp, which correspond to adaptor-ligated constructs derived from the 21 and 30 nucleotide RNA fragments, respectively. DO NOT cut the 89 bp band out, as this is adaptor dimer. (Figure 1).

Figure 1: Small RNA Libraries



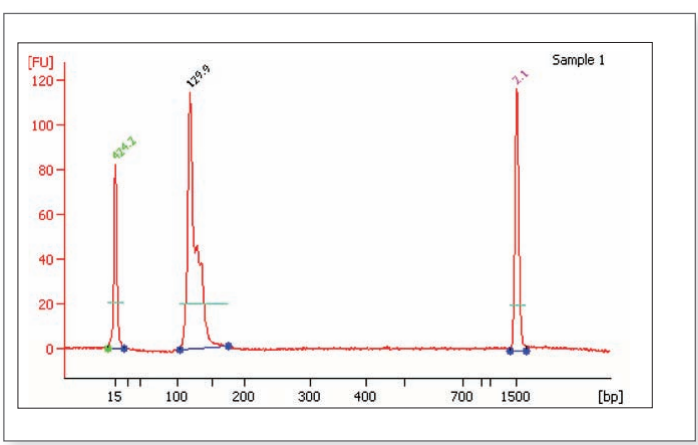
Small RNA libraries generated from 5 µg Human Brain Total RNA (Lane 2), 5 µg Rat Testis Total RNA (Lane 3) and 5 µl of the Quick-Load pBR322 DNA-MspI Digest (Lane 1). The 110 nucleotide band in lane 1 contains prepared miRNA library generated from ~21 nucleotide small RNA fragments. The 110 nucleotide band in lane 2 and 119 nucleotide band in lane 3 contain prepared piwi-interacting RNAs (piRNAs), microRNAs and other regulatory small RNA molecule libraries generated from ~20 and ~30 nucleotide RNA fragments. The 89 nucleotide band in both lanes contains adapter dimer.

Gel Purification of Amplified cDNA Library

7. Place the gel slice in a 1.5 ml tube, crush the gel slice with the RNase-free Disposable Pellet Pestles and soak in 100 µl 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, and centrifuge the filter for 2 minutes at 14,000 rpm.
10. Check the size (should be between 110-130 bp), purity and concentration on an Agilent 2100 Bioanalyzer® (Agilent Technologies, Inc.) using a DNA high sensitivity on a DNA 1000 CHiP. If the gel doesn't run properly for your sample perform the following ethanol precipitation.
11. Recover eluate, add 1 µl Linear Acrylamide, 25 µl 3M sodium acetate pH 5.2 and 750 µl of 100% ethanol and vortex well.
12. Precipitate in a dry ice/methanol bath for at least 30 minutes then spin in a microcentrifuge (>14.000 x g) at 4°C for 30 minutes.

13. Remove the supernatant taking care not to disturb/remove the pellet and wash the pellet with 80% ethanol by vortexing vigorously.
14. Spin in a microcentrifuge (>14.000 x g) at 4°C for 30 minutes.
15. Air dry pellet for up to 10 minutes at room temperature to remove residual ethanol.
16. Resuspend pellet in 10 µl TE Buffer. Perform the following quality control analysis on your sample library to quantify the DNA concentration.
17. Load 1 µl of the reconstituted construct on an Agilent 2100 Bioanalyzer using a DNA High Sensitivity or an Agilent DNA-1000 chip (Figure 2).
18. Check the size (should be ~110–119 bp), 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.

Figure 2: Agilent Bioanalyzer Trace of a final Human Brain miRNA Library showing a 129.9 nM peak between 110 and 119 bp.



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 $MgCl_2$
1 mM DTT
12.5% Polyethylene glycol MW (PEG 8000)
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 with1 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 $MgCl_2$) 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 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$_f$
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 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 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 with1 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 $MgCl_2$) 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: 200 units of T4 RNA Ligase 2, truncated$_f$ 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 $MgCl_2$, 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 3

#E6161A: 15 µl
#E6161AA: 50 µl

Store at –20°C

Description: 5´ adenylated, 3´ blocked oligodeoxynucleotide

Sequence: rAppCGCCTTGGCCGTACAGCAG-NH2-3´

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1 µl NEBNext 3´ SR Adaptor 3 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 SR Adaptor 3 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 3 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 3 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 $MgCl_2$) 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: NEBNext 3´ SR Adaptor 3 purity is determined by HPLC to be > 99%.

Lot Controlled

NEBNext 5´ SR Adaptor 3

#E6162A: 750 pmol
#E6162AA: 3,750 pmol

Store at –20°C

Description: Single-Stranded oligoribonucleotide (23 nucleotide length)

Sequence: 5´-rCrCrUrCrUrCrUrArUrGrGrGrCrArGrUrCrGrGrUrGrArU-3´

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1 µl NEBNext 5´ SR Adaptor 3 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 3 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 3 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 3 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 3 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.

HPLC: NEBNext 5´ SR Adaptor 3 purity is determined by HPLC to be > 99%.

Lot Controlled

NEBNext 5´ Ligation Reaction Buffer

#E6126A: 15 µl **Concentration: 10X**
#E6126AA: 50 µl

Store at –20°C

1X NEBNext 5´ Ligation Reaction Buffer:
50 mM Tris-HCl
10 mM $MgCl_2$
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 with1 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 $MgCl_2$) 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 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 $MgCl_2$) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

Functional Activity: One unit of T4 RNA Ligase 1 is defined as the amount of enzyme required to convert 1 nanomole of 5´-$[_{32}P]rA_{16}$ into a Phosphatase-resistant form in 30 minutes at 37°C.

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 SR RT Primer 3

#E6163A: 15 µl
#E6163AA: 50 µl

Store at –20°C

Description: Single-stranded oligodeoxynucleotide (19 nucleotide length)

Sequence: 5´-CTGCTGTACGGCCAAGGCG-3´

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1 µl NEBNext SR RT Primer 3 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 3 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 3 with1 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 3 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 3 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 First Strand Synthesis Reaction Buffer

#E6169A: 0.04 ml **Concentration: 5X**
#E6169AA: 0.2 ml

Store at –20°C

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

Quality Control Assays

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

Endonuclease Activity: Incubation of a 50 µl reaction containing NEBNext First Strand Synthesis 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 NEBNext First Strand Synthesis Reaction Buffer with 40 ng of RNA transcript for 16 hours at 37°C resulted in no detectable degradation of RNA as determined by gel electrophoresis.

Phosphatase Activity: Incubation of 20 µl NEBNext First Strand Synthesis Reaction Buffer in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

Lot Controlled

M-MuLV Reverse Transcriptase (RNase H$^-$)

#E6168A: 0.015 ml **Concentration: 200,000 U/ml**
#E6168AA: 0.05 ml

Store at –20°C

Description: M-MuLV Reverse Transcriptase (RNase H$^-$) is a recombinant M-MuLV reverse transcriptase with reduced RNase H activity and increased thermostability. It can be used to synthesize first strand cDNA at higher temperatures than the wild type M-MuLV. The enzyme is active up to 50°C, providing higher specificity, higher yield of cDNA and more full-length cDNA product up to 12 kb.

Source: The gene encoding a mutant M-MuLV Reverse Transcriptase (RNase H$^-$) is expressed in *E. coli* and purified to near homogeneity.

Supplied in: 20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01% (v/v) IGEPAL® CA-630, 50% (v/v) glycerol

Quality Control Assays

16-Hour Incubation: A 50 µl reaction containing 1 µg of ϕX174 DNA and 100 units of M-MuLV Reverse Transcriptase (RNase H$^-$) incubated for 16 hours at 37°C resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Exonuclease Activity: Incubation of a 50 µl reaction containing 100 units of M-MuLV Reverse Transcriptase (RNase H$^-$) with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA (10^5 cpm/µg) for 4 hours at 37°C released < 0.2% of the total radioactivity.

RNase Activity: Incubation of a 10 µl reaction containing 100 units of M-MuLV Reverse Transcriptase (RNase H$^-$) with 40 ng of RNA transcripts for 2 hours at 37°C resulted in no detectable degradation of the RNA as determined by gel electrophoresis.

Phosphatase Activity: Incubation of 100 units of M-MuLV Reverse Transcriptase (RNase H$^-$) in protein phosphatase assay buffer 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.

Protein Purity (SDS-PAGE): M-MuLV Reverse Transcriptase (RNase H$^-$) is > 95% pure as determined by SDS PAGE analysis using Coomassie blue detection.

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 [^{3}H] *E. coli* DNA (20^5 cpm/µg) for 4 hours at 37°C released < 0.5% of the total radioactivity.

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

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

Endonuclease Activity: Incubation of a 10 µl reaction containing 40 units of Murine RNase Inhibitor with 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 $MgCl_2$) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

Lot Controlled

References:

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

One*Taq* Hot Start 2X Master Mix with Standard Buffer

E6167A: **0.25 ml** **Concentration: 2X**
E6167AA: **1.25 ml**

Store at –20°C

1X One*Taq* Hot Start 2X Master Mix with Standard Buffer:
20 mM Tris-HCl
1.8 mM $MgCl_2$
22 mM NH_4Cl_2
22 mM KCl
0.2 mM dNTPs
5% Glycerol
0.06% IGEPAL CA-630
0.05% Tween-20
25 units/ml One*Taq* Hot Start DNA Polymerase

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing One*Taq* Hot Start 2X Master Mix with Standard Buffer at 1X concentration 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 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.

Endonuclease Activity: Incubation of a minimum of 10 µl of One*Taq* Hot Start 2X Master Mix with Standard Buffer at a 1X concentration with 1 µg of ϕX174 RF I DNA in assay buffer for 4 hours at 37°C in 50 µl reactions results in < 10% conversion to RF II as determined by agarose gel electrophoresis.

RNase Activity: Incubation of a 10 µl reaction of One*Taq* Hot Start 2X Master Mix with Standard Buffer at a 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 a minimum of 10 µl of One*Taq* Hot Start 2X Master Mix with Standard Buffer at a 1X concentration in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

Functional Activity: 5 kb Lambda PCR: 25 cycles of PCR amplification of 5 ng Lambda DNA with 1X One*Taq* Hot Start Master Mix with 1X One*Taq* Hot Start Master Mix with Standard Buffer in a 25 µl reaction in the presence of 0.2 µM primers results in the expected 5 kb product.

Lot Controlled

NEBNext Index 1 Primer for SOLiD

#E6164A: 0.025 ml
#E6164AA: 0.125 ml

Store at –20°C

Sequence:
5´-CTGCCCCGGGTTCCTCATTCTCTGTGTAAGAGGCTGCTGTACGGCCAAGGCG-3´

Quality Control Assays

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

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

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

Phosphatase Activity: Incubation of NEBNext Index [X] Primer in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) 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.

NEBNext SR Primer R3

#E6165A: 0.025 ml
#E6165AA: 0.125 ml

Store at –20°C

Sequence: 5´-CCACTACGCCTCCGCTTTCCTCTCTATGGGCAGTCGGTGAT-3´

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1 µl NEBNext SR Primer R3 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 R3 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 R3 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 R3 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 R3 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

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 poylacrylamide 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
0.015% 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 $MgCl_2$) 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-Load pBR322 DNA-MspI Digest

#E6166A: 0.1 ml **Concentration: 100 µg/ml**
#E6166AA: 0.5 ml

Store at 4°C or –20°C

Description: Quick-Load pBR322 DNA-MspI Digest is a pre-mixed, ready-to-load molecular weight marker containing bromophenol blue as a tracking dye. The Msp I Digest of pBR322 DNA yields 26 fragments.

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 pBR322 DNA-MspI Digest 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 pBR322 DNA-MspI Digest 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 pBR322 DNA-MspI Digest with1 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 pBR322 DNA-MspI Digest 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 pBR322 DNA-MspI Digest 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

DNA Gel Elution Buffer

#E6134A: 5 ml **Concentration: 1X**
#E6134AA: 25 ml

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 $MgCl_2$) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

Lot Controlled

Linear Acrylamide

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

Store at –20°C or 4°C

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

Quality Control Assays

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

Endonuclease Activity: Incubation of a 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 $MgCl_2$) 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

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 with1 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 $MgCl_2$) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

Lot Controlled

Nuclease-free Water

#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 1X Nuclease-free Water 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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