Appendix X

Technical Information

BamH I

R0136L
50,000 units 20,000 U/ml Lot: 91
RECOMBINANT Store at −20°C

Recognition Site:
5'...GGATCC...3'
3'...CC TAGG...5'

Source: An E. coli strain that carries the cloned BamH I gene from Bacillus amyloliquefaciens H (ATCC 49783)

Supplied in: 50 mM KCI, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 μg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer BamH I, 100X BSA.

Reaction Conditions: 1X NEBuffer BamH I, supplemented with 100 μg/ml BSA. Incubate at 37°C.

1X NEBuffer BamH I:
150 mM NaCl
10 mM Tris-HCl
10 mM MgCl₂
1 mM dithiothreitol
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 μg of λ DNA in 1 hour at 37°C in a total reaction volume of 50 μl.

Diluent Compatibility: Diluent Buffer A
50 mM KCI, 10 mM Tris-HCl, 1 mM EDTA, 1 mM dithiothreitol, 200 μg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Control Assays
Ligation: After 50-fold overdigestion with BamH I, > 95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5’ terminus concentration of 1–2 μM) at 16°C. Of these ligated fragments, > 95% can be resut.

16-Hour Incubation: A 50 μl reaction containing 1 μg of DNA and 100 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme.

Exonuclease Activity: Incubation of 100 units of enzyme with 1 μg sonicated [3H] DNA (10³ cpm/μg) for 4 hours at 37°C in 50 μl reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 100 units of enzyme with 1 μg pX174 RF I DNA for 4 hours at 37°C in 50 μl reaction buffer resulted in < 10% conversion to RF II.

Blue/White Screening Assay: This enzyme has been tested to determine the integrity of the DNA ends produced after digestion with an excess of enzyme. An appropriate vector is digested at a unique site within lacZ gene with a 10-fold excess of enzyme, ligated, transformed and plated on Xgal/ IPTG/Amp plates. Successful expression of β-galactosidase is a function of how intact its gene remains after cloning, an intact gene gives rise to a blue colony, an interrupted gene (i.e. degraded DNA end) gives rise to a white colony. Enzymes must produce fewer than 3% white colonies to be Blue/White Certified.

Enzyme Properties
Activity in NEBuffers:
NEBuffer 1 75%
NEBuffer 2 100%
NEBuffer 3 50%
NEBuffer 4 75%

These buffers are not recommended because of the likelihood of star activity.

Survival in a Reaction: A minimum of 0.50 unit is required to digest 1 μg of substrate DNA in 16 hours.

Heat Inactivation: No

Plasmid Cleavage: Number of units required to cleave 1 μg of supercoiled plasmid DNA in one hour: pUC19 ~ 1 unit, pBR322 ~ 3 units, LITMUS ~ 5 units.

Notes: Not sensitive to dam, dcm or mammalian CpG methylation.

Conditions of low ionic strength, high enzyme concentration, glycerol concentration > 5%, or pH > 8.0 may result in star activity.

U.S. Patent No. 5,137,823

(See other side)
Appendix 10

**Bgl II**

1-800-352-7799
info@neb.com
www.neb.com

R0144L

10,000 units 10,000 U/ml Lot: 39
RECOMBINANT STORE AT −20°C

**Recognition Site:**

5‘...AGATCT...3’
3‘...TCAGA...5’

**Source:** An *E. coli* strain that carries the cloned Bgl II gene from *Bacillus globigii* (ATCC 49760)

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 200 μg/ml BSA and 50% glycerol.

**Reagents Supplied with Enzyme:**

10X NEBuffer 3.

**Reaction Conditions:** 1X NEBuffer 3. Incubate at 37°C.

**1X NEBuffer 3:**

100 mM NaCl
50 mM Tris-HCl
10 mM MgCl₂
1 mM DTT
pH 7.9 @ 25°C

**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 μg of λ DNA in 1 hour at 37°C in a total reaction volume of 50 μl.

**Diluent Compatibility:** Diluent Buffer A

50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 200 μg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

**Quality Control Assays**

**Ligation:** After 40-fold overdigestion with Bgl II, >95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5 termini concentration of 1–2 μM) at 16°C. Of these ligated fragments, >95% can be recut.

**16-Hour Incubation:** A 50 μl reaction containing 1 μg of DNA and 100 units of Bgl II incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme.

**Exonuclease Activity:** Incubation of 300 units of Bgl II with 1 μg sonicated ³H DNA (10⁵ cpm/μg) for 4 hours at 37°C in 50 μl reaction buffer released < 0.1% radioactivity.

**Endonuclease Activity:** Incubation of 80 units of Bgl II with 1 μg of pX174 RF I DNA for 4 hours at 37°C in 50 μl reaction buffer resulted in 10% conversion to RF II.

**Blue/White Screening Assay:** This enzyme has been tested to determine the integrity of the DNA ends produced after digestion with an excess of enzyme. An appropriate vector is digested at a unique site within the lacZα gene with a 10-fold excess of enzyme, ligated, transformed and plated on XGal/IPTG/Amp plates. Successful expression of β-galactosidase is a function of how intact its gene remains after cloning, an intact gene gives rise to a blue colony, an interrupted gene (i.e. degraded DNA end) gives rise to a white colony. Enzymes must produce fewer than 3% white colonies to be Blue/White Certified.

**Enzyme Properties**

**Activity in NEBuffer:**

NEBuffer 1 10%
NEBuffer 2 75%
NEBuffer 3 **100%**
NEBuffer 4 10%

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

**Survival in a Reaction:** A minimum of 0.25 unit is required to digest 1 μg of substrate DNA in 16 hours.

**Heat Inactivation:** No

**Note:** Not sensitive to dam, dcm or mammalian CpG methylation.

U.S. Patent No. 5,434,068

CERTIFICATE OF ANALYSIS
Technical Information

EcoRI

R0101L
50,000 units (Supplied as 2 tubes of 25,000 units)
20,000 U/ml
Lot: 31
RECOMBINANT
Store at −20°C

Recognition Site:
5'...GAAATTCC...3'
3'...CTTAAG...5'

Source: An E. coli strain that carries the cloned EcoRI gene from E. coli/RY13 (R. N. Yoshimori)

Supplied in: 300 mM NaCl, 10 mM KPO4, (pH 7.5), 0.1 mM EDTA, 10 mM 2-mercaptoethanol, 0.15% Triton X-100, 200 μg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer EcoRI I.

Reaction Conditions: 1X NEBuffer EcoRI I.
Incubate at 37°C.

1X NEBuffer EcoRI I:
50 mM NaCl
100 mM Tris-HCl
10 mM MgCl2
0.025% Triton X-100
pH 7.5 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 μg of λ DNA in 1 hour at 37°C in a total reaction volume of 50 μl.

Diluent Compatibility: Diluent Buffer C
250 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 0.15% Triton X-100, 200 μg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Heat Inactivation: 250 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Quality Control Assays
Ligation: After 10-fold overdigestion with EcoRI, >95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5’ termini concentration of 1–2 μM) at 16°C. Of these ligated fragments, >95% can be recut.

16-Hour Incubation: A 50 μl reaction containing 1 μg of DNA and 100 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme.

Exonuclease Activity: Incubation of 200 units of enzyme with 1 μg sonicated [3H] DNA (100 cpm/μg) for 4 hours at 37°C in 50 μl reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 100 units of enzyme with 1 μg pX174 RF I DNA for 4 hours at 37°C in 50 μl reaction buffer resulted in no detectable conversion to RF II.

Blue/White Screening Assay: This enzyme has been tested to determine the integrity of the DNA ends produced after digestion with an excess of enzyme. An appropriate vector is digested at a unique site within lacZ gene with a 10-fold excess of enzyme, ligated, transformed and plated on XG6/IPTG/Amp plates. Successful expression of β-galactosidase is a function of how intact its gene remains after cloning, an intact gene gives rise to a blue colony, an interrupted gene (i.e. degraded DNA end) gives rise to a white colony. Enzymes must produce fewer than 3% white colonies to be Blue/White Certified.

Enzyme Properties
Activity in NEBuffers:
NEBuffer 1 100%
NEBuffer 2 100%
NEBuffer 3 100%
NEBuffer 4 100%

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

Survival in a Reaction: A minimum of 0.13 unit is required to digest 1 μg of substrate DNA in 16 hours.

Plasmid Cleavage: Number of units required to cleave 1 μg of supercoiled plasmid DNA in one hour: pLITMUS = 4 units, pBR322 = 2.5 units, pUC19 = 2.5 units.

Notes: Cleavage of mammalian genomic DNA is impaired by overlapping Cpg methylation.

Conditions of low ionic strength, high enzyme concentration, glycerol concentration > 5% or pH > 8.0 may result in star activity.
**Hind III**

50,000 units  20,000 U/ml  Lot: 60

**RECOMBINANT**  Store at -20°C

**Recognition Site:**

5' ... AATGCTT ... 3'

3' ... TTGCAAA ... 5'

**Source:** An *E. coli* strain that carries the cloned Hind III gene from *Haemophilus influenzae* Rd (ATCC 51907)

Supplied in: 250 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 500 μg/ml BSA and 50% glycerol.

**Reagents Supplied with Enzyme:**

10X NEBuffer 2

**Reaction Conditions:** 1X NEBuffer 2

Incubate at 37°C.

1X NEBuffer 2:

50 mM NaCl

10 mM Tris-HCl

10 mM MgCl₂

1 mM DTT

pH 7.9 @ 25°C

**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 μg of λ DNA in 1 hour at 37°C in a total reaction volume of 50 μl.

**Diluent Compatibility:** Diluent Buffer B

300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 500 μg/ml BSA and 50% glycerol. (pH 7.4 @ 25°C).

**Quality Control Assays**

**Ligation:** After 200-fold overdigestion with Hind III, >95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5' terminus concentration of 1–2 μM) at 16°C. Of these ligated fragments, >95% can be recut.

**16-Hour Incubation:** A 50 μl reaction containing 1 μg of DNA and 400 units of enzyme incubated for 16 hours resulted in no degradation of the DNA bands due to nonspecific nuclease. However, fragments produced by noncanonical cleavage due to star activity may be observed with 80 units of enzyme in similar conditions.

**Exonuclease Activity:** Incubation of 2,000 units of enzyme with 1 μg sonicated [3H] DNA (10⁶ cpm/μg) for 4 hours at 37°C in 50 μl reaction buffer released < 0.1% radioactivity.

**Endonuclease Activity:** Incubation of 40 units of enzyme with 1 μg λX174 RF I DNA for 4 hours at 37°C in 50 μl reaction buffer resulted in < 20% conversion to RF II.

**Blue/White Screening Assay:** This enzyme has been tested to determine the integrity of the DNA ends produced after digestion with an excess of enzyme. An appropriate vector is digested at a unique site within lacZ gene with a 10-fold excess of enzyme, ligated, transformed and plated on XGal/IPTG/Amp plates. Successful expression of β-galactosidase is a function of how intact its gene remains after cloning, an intact gene gives rise to a blue colony, an interrupted gene (i.e. degraded DNA end) gives rise to a white colony. Enzymes must produce fewer than 3% white colonies to be Blue/White Certified.

**Enzyme Properties**

**Activity in NEBuffers:**

NEBuffer 1  50%

NEBuffer 2  100%

NEBuffer 3  10%

NEBuffer 4  50%

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

**Survival in a Reaction:** A minimum of 0.13 unit is required to digest 1 μg of substrate DNA in 16 hours.

**Heat Inactivation:** 100 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

**Notes:** Not sensitive to dam, dcm or mammalian CpG methylation.

Conditions of high enzyme concentration, glycerol concentration > 5% or pH > 8.0 may result in star activity.

U.S. Patent No. 5,180,673  CERTIFICATE OF ANALYSIS
Technical Information

Technical Information

Pst I

R0140L

50,000 units 20,000 U/ml Lot: 40
RECOMBINANT Store at –20°C

Recognition Site:
5′...CTGCAAG...3′
3′...GACGTCA...5′

Source: An E. coli strain that carries the cloned Pst I gene from Providencia stuartii 164 (ATCC 49762)

Supplied in: 200 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 0.15% Triton X-100, 200 μg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 3, 100X BSA.

Reaction Conditions: 1X NEBuffer 3, supplemented with 100 μg/ml BSA. Incubate at 37°C.

1X NEBuffer 3:
100 mM NaCl
50 mM Tris-HCl
10 mM MgCl₂
1 mM DTT
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 μg of λ DNA in 1 hour at 37°C in a total reaction volume of 50 μl.

Diluent Compatibility: Diluent Buffer C 250 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 0.15% Triton X-100, 200 μg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Control Assays
Ligation: After 200-fold overdigestion with Pst I, > 95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5′ termini concentration of 1–2 μM) at 16°C. Of these ligated fragments, > 95% can be recut.

16-Hour Incubation: A 50 μl reaction containing 1 μg of DNA and 400 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as the reaction incubated for 1 hour with 1 unit of enzyme.

Exonuclease Activity: Incubation of 400 units of enzyme with 1 μg sonicated 32P DNA (10⁶ cpm/μg) for 4 hours at 37°C in 50 μl reaction buffer released < 0.1% radioactivity.

Blue/White Screening Assay: This enzyme has been tested to determine the integrity of the DNA ends produced after digestion with an excess of enzyme. An appropriate vector is digested at a unique site within lacZα gene with a 10-fold excess of enzyme, ligated, transformed and plated on XGα/IPTG/Amp plates. Successful expression of β-galactosidase is a function of how intact its gene remains after cloning, an intact gene gives rise to a blue colony, an interrupted gene (i.e. degraded DNA end) gives rise to a white colony. Enzymes must produce fewer than 3% white colonies to be Blue/White Certified.

Enzyme Properties
Activity in NEBuffers:
NEBuffer 1 75%
NEBuffer 2 75%
NEBuffer 3 100%
NEBuffer 4 50%

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

Survival in a Reaction: A minimum of 0.50 unit is required to digest 1 μg of substrate DNA in 16 hours.

Heat Inactivation: 80°C for 20 minutes.

Plasmid Cleavage: Number of units required to cleave 1 μg of supercoiled plasmid DNA in one hour: pUC19 = 1 unit, pBR322 = 1 unit, LITMUS = 1 unit.

Note: Not sensitive to dam, dcm or mammalian CpG methylation.

Certificate of Analysis
Xba I

15,000 units  20,000 U/ml  Lot: 34
RECOMBINANT  Store at ~20°C

Recognition Site:
5′... TCTAGA ...3′
3′... AGATCT ...5′

Source: An E. coli strain that carries the cloned Xba I gene from Xanthomonas badlii (ATCC 11672)

Supplied in: 50 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 200 μg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 2, 100X BSA.

Reaction Conditions: 1X NEBuffer 2, supplemented with 100 μg/ml BSA. Incubate at 37°C.

1X NEBuffer 2:
- 50 mM NaCl
- 10 mM Tris-HCl
- 10 mM MgCl₂
- 1 mM DTT
- pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 μg of λ DNA (dam/Hind III digest) in 1 hour at 37°C in a total reaction volume of 50 μl.

Diluent Compatibility: Diluent Buffer A 50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 200 μg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

Quality Control Assays
Ligation: After 20-fold overdigestion with Xba I, >95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5′ termini concentration of 1–2 mM) at 16°C. Of these ligated fragments, >95% can be recut.

16-Hour Incubation: A 50 μl reaction containing 1 μg of DNA and 200 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme.

Exonuclease Activity: Incubation of 200 units of enzyme with 1 μg sonicated 3H DNA (10⁴ cpm/μg) for 4 hours at 37°C in 50 μl reaction buffer released 0.15% radioactivity.

Endonuclease Activity: Incubation of 200 units of enzyme with 1 μg pX174 RF I DNA for 4 hours at 37°C in 50 μl reaction buffer resulted in 10% conversion to RF II.

Blue/White Screening Assay: This enzyme has been tested to determine the integrity of the DNA ends produced after digestion with an excess of enzyme. An appropriate vector is digested at a unique site within lacZ gene with a 10-fold excess of enzyme, ligated, transformed and plated on XGal/IPTG/Amp plates. Successful expression of β-galactosidase is a function of how intact its gene remains after cloning, an intact gene gives rise to a blue colony, an interrupted gene (i.e. degraded DNA end) gives rise to a white colony. Enzymes must produce fewer than 3% while colonies to be Blue/White Certified.

Survival in a Reaction: A minimum of 0.13 unit is required to digest 1 μg of substrate DNA in 16 hours.

Enzyme Properties
Activity in NEBuffers:
- NEBuffer 1  0%
- NEBuffer 2  100%
- NEBuffer 3  75%
- NEBuffer 4  75%

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

Heat Inactivation: 200 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Plasmid Cleavage: Number of units required to cleave 1 μg of supercoiled plasmid DNA in one hour: pUC 19 = 2 units.

Note: Blocked by overlapping dam methylation.
\textbf{T4 DNA Ligase}

\textbf{Cat. no. 15224-017}
\textbf{Cat. no. 15224-025}

\textbf{Conc.}: 1 U/\mu l
\textbf{Size}: 100 units
\textbf{Size}: 500 units

\textbf{Store at} -20°C in a non-frost-free freezer.

\textbf{Note}: T4 DNA Ligase is unstable on ice for long periods. Therefore, Invitrogen recommends the enzyme be kept at -20°C until within 5-10 minutes of use and returned IMMEDIATELY to -20°C after use.

\textbf{Description}: T4 DNA Ligase can be used to join DNA fragments with staggered or stranded DNA having 3'-hydroxyl and 5'-phosphate ends. The enzyme is isolated from Escherichia coli lambda hig4896.

\textbf{Unit Definition}: One (Weiss) unit catalyzes the exchange of 1 mmol of ATP into [\gamma-32P]ATP in 20 minutes at 37°C (1).

\textbf{Components}: T4 DNA Ligase
5X DNA Ligase Reaction Buffer

\textbf{Buffer Composition}:
- 10 mM Tris-HCl (pH 7.5)
- 50 mM KCl
- 1 mM DTT
- 50% (v/v) glycerol

\textbf{Store buffer at} -20°C.

\textbf{Concentrations}:
- 20 mM Tris-HCl (pH 7.6)
- 500 mM KCl
- 5 mM ATP
- 25% (v/v) polyethylene glycol-8000.

\textbf{Part no.} 15224 ppm \textbf{Rev. date}: 20 May 2002

\textbf{Quality Control}:
This product has passed the following quality control assays: functional absence of exonuclease and endonuclease activities, ligation/resolv and ligation efficiency.

The enclosed buffers were assayed with the enzyme and met quality control specifications.

\textbf{Protocols}:
\textbf{Note}: Before use, thaw 5X DNA Ligase Reaction Buffer at room temperature and vortex vigorously to dissolve any precipitated material.

\textbf{Recommended Conditions for General Cloning and Library Construction}:

| Insert: Vector Molar Ratio | 3:1 |
| Vector Ends | 3-30 fmol | 15-60 fmol |
| Insert Ends | 9-30 fmol | 45-100 fmol |
| Total DNA | 0.01-0.1 pg | 0.1-0.0 pg |
| T4 DNA Ligase | 0.1 unit | 1.0 unit |
| Autoclaved distilled water | 20 \mu l | 20 \mu l |
| Temperature | 23-26°C | 14°C |
| Time | 1 h | 16-24 h |

\textbf{Note}: For optimal transformation, dilute the ligation reaction 5-fold, to at least 100 \mu l, prior to adding to competent cells (2).
Appendix 10

M0223S

100 units 4,000 U/ml  Lot: 0081306
RECOMBINANT Store at -20°C  Exp: 6/15

Methylation Site: $\text{CH}_3$

5'...GGATCC...3'

3'...CCTAGG...5'

$\text{CH}_3$

Description: BamHI Methyltransferase modifies the internal cytosine residue (N^1) in the sequence above.

Source: An E. coli strain that carries the cloned BamHI modification gene from Bacillus amyloquefaciens H (ATCC 49763)

Supplied in: 50 mM Tris-HCl (pH 7.5), 10 mM EDTA, 1 mM DTT, 200 μg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X BamHI Methyltransferase Reaction Buffer, 400X S-adenosylmethionine (32 mM)

Reaction Conditions: 1X BamHI Methyltransferase Reaction Buffer, 80 μM S-adenosylmethionine. Incubate at 37°C.

1X BamHI Methyltransferase Reaction Buffer:
50 mM Tris-HCl
10 mM EDTA
5 mM DTT
pH 7.5 @ 25°C

Protection Assay Conditions: BamHI Methyltransferase is incubated with 1 μg $\lambda$ DNA in 10 μl 1X BamHI Methyltransferase Reaction Buffer, supplemented with 80 μM S-adenosylmethionine, for one hour at 37°C followed by 15 minutes at 65°C. The extent of protection by BamHI Methyltransferase is determined by the addition of 40 μl NEBuffer 1 supplemented with 10 mM MgCl₂ and 10 units of BamHI restriction endonuclease. Incubation at 37°C for 30 minutes is followed by analysis on agarose gels.

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 μg $\lambda$ DNA in 1 hour at 37°C in a total reaction volume of 10 μl against cleavage by BamHI restriction endonuclease.

Quality Control Assays

Exonuclease Activity: Incubation of 30 units of BamHI Methyltransferase with 1 μg sonicated $^{3}H$ DNA (10⁶ cpm/μg) for 4 hours at 37°C in 50 μl NEBuffer 2 [50 mM NaCl, 10 mM Tris-HCl (pH 7.9 @ 25°C), 10 mM MgCl₂, 1 mM DTT] released < 0.3% of the total radioactivity.

Endonuclease Activity: Incubation of 15 units with 1 μg of $\lambda$ HindIII DNA fragments in a 50 μl NEBuffer 2 for 16 hours at 37°C resulted in no degradation of the DNA as determined by visualization of clear and sharp bands following gel electrophoresis.

Storage of SAM: S-adenosylmethionine or SAM is stored at -20°C as a 32 mM solution dissolved in 0.005 M sulfuric acid and 10% ethanol. Under these conditions SAM is stable for up to 6 months. SAM is unstable at (pH 7.5), 37°C, (1) and should be replenished in reactions incubated longer than 4 hours.

Methylation can be optimized by using fresh SAM.

Reference:

Companion Product:
S-adenosylmethionine (SAM) #B9003S 0.5 ml

Certificate of Analysis

156
Technical Information

EcoRI Methyltransferase

M0211S

10,000 units 40,000 U/ml Lot: 0121304
RECOMBINANT Store at –20°C Exp: 4/15

Methylation Site:

\[ \text{CH}_3 \]

5': G A T T C ... 3'

3': C T T A A G ... 5'

Description: EcoRI Methyltransferase modifies the internal adenine residue (N) in the sequence above.

Source: An E. coli strain that carries the cloned EcoRI modification gene from Escherichia coli RY13 (R.N. Yoshimori)

Supplied in: 200 mM NaCl, 100 mM KPO\(_4\), (pH 7.4), 0.1 mM EDTA, 10 mM 2-mercaptoethanol, 200 µg/ml BSA, and 50% glycerol.

Reagents Supplied with Enzyme:

10X EcoRI Methyltransferase Reaction Buffer, 400X S-adenosylmethionine (32 mM).

Reaction Conditions: 1X EcoRI Methyltransferase Reaction Buffer, supplemented with 80 µM S-adenosylmethionine (supplied). Incubate at 37°C.

1X EcoRI Methyltransferase Reaction Buffer:

50 mM NaCl
50 mM Tris-HCl
10 mM EDTA
pH 8.0 @ 25°C

Protection Assay Conditions: EcoRI Methyltransferase is incubated with 1 µg of λ DNA in 10 µl 1X EcoRI Methyltransferase Reaction Buffer, supplemented with 80 µM S-adenosylmethionine, for one hour at 37°C followed by 15 minutes at 65°C. The extent of protection by EcoRI Methyltransferase is determined by the addition of 40 µl NEBuffer 2 and 5 units of EcoRI restriction endonuclease. Incubation at 37°C for 30 minutes is followed by analysis on an agarose gel.

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg of λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by EcoRI restriction endonuclease.

Quality Control Assays

16-Hour Incubation: A 50 µl reaction containing 1 µg of HindIII digested λ DNA and 1,500 units of EcoRI Methyltransferase incubated for 16 hours at 37°C in NEBuffer 2 resulted in no detectable degradation.

Exonuclease Activity: Incubation of 4,000 units of EcoRI Methyltransferase with 1 µg sonicated [\(^{3}H\) DNA (10\(^6\) cpm/µg) for 4 hours at 37°C in 50 µl NEBuffer 2 (50 mM NaCl, 10 mM Tris-HCl (pH 7.9 @ 25°C), 10 mM MgCl\(_2\), 1 mM DTT) released 0.3% of the total radioactivity.

Storage of SAM: S-adenosylmethionine (SAM) is stored at –20°C as a 32 mM solution dissolved in 0.005 M sulfuric acid and 10% ethanol. Under these conditions SAM is stable for up to 6 months. SAM is unstable at (pH 7.5), 37°C, (1) and should be replenished in reactions incubated longer than 4 hours.

Methylation can be optimized by using fresh SAM.

Note: EcoRI Methyltransferase is inhibited by MgCl\(_2\).

Only 50% activity is retained at a concentration of 4 mM MgCl\(_2\).

Reference:


Companion Product:

S-adenosylmethionine (SAM) #B9063S 0.5 ml

Certificate of Analysis

157
Bacterial Alkaline Phosphatase

Cat. No. 18011-015
Lot No. KAK411
Exp. Date: 03/06
Store at -20°C (except from here).

Description:
Bacterial Alkaline Phosphatase (BAP) is a phosphomonoesterase which
hydrolyzes 3' and 5' phosphates from DNA and RNA. It is suitable for removing
5' phosphates prior to end-labeling and for dephosphorylating vector prior to
insert ligation. BAP is active at 65°C for at least 1 h and can be inactivated by
phosphatase extraction. It is sensitive to inhibition by micromolar amounts of
inorganic phosphate. The enzyme is purified from E. coli C90.

Components:
18011-015 BAP
Y02290 10X Dephosphorylation Buffer
Lot No. KAK411 Lot No. KAK412

Unit Definition:
One unit hydrolyzes 1 nmol of ATP in 30 min at 37°C.

Storage Buffer:
10X Dephosphorylation Buffer:
10 mM Tris-HCl (pH 8.0)
120 mM NaCl
50% (v/v) glycerol
Store buffer at 4°C or -20°C.

Quality Control Assays:
This protocol has passed the following quality control assays: absence of
detectable endonuclease, exonuclease and ribonuclease activities; performance in
dehphosphorylating blunt-ended DNA. The enclosed buffers were assayed with the enzyme and met quality control specifications.

Dephosphorylation of DNA
This protocol is suitable for dephosphorylating 1 pmole of 5' DNA termini in a
100 µl reaction volume. The number of 5' termini can be calculated as follows:

\[ \text{mol of termini of double-stranded DNA} = 2 \times (\text{g of DNA}) \times \frac{\text{molecular weight of DNA (Da)}}{1000} \times \left( \frac{1}{\text{number of bp}} \right) \times 600 \text{ Da/bp} \]

1. Dilute BAP to 7 units/µl with 1X Dephosphorylation Buffer.
2. Add the following to a microcentrifuge tube:
   - 5' DNA termini (1 pmole)
   - 10X Dephosphorylation buffer
   - Autoclaved distilled water up to 50 µl
   - Add 10 µl of diluted enzyme (70 units) of BAP.
3. Incubate 65°C for 60 min.
4. Stop the reaction by adding 1 µl of 0.5 M EDTA (pH 8.0) and heat to
   50°C for 10 min.
5. Extract the reaction mixture with equal volume (100 µl) of buffer-
saturated phenol. Centrifuge for 1 min to separate the phases.
6. Transfer the upper aqueous phase to a new microcentrifuge tube.
7. Extract the aqueous phase with an equal volume of chloroform/isoamyl
   alcohol (24:1 v/v). Centrifuge for 1 min to separate the phases.
8. Transfer the upper aqueous phase to a new microcentrifuge tube.
9. Ethanol precipitate the DNA by adding 0.5 volumes of 7.5 M ammonium
   acetate followed by 2.5 volumes of ethanol. Centrifuge at 15,000 x g for
   30 min.
10. Dissolve pellet in the buffer appropriate for the next reaction.

Dec. Rev. 5/04/05

This product is distributed for laboratory research use only. CAUTION: Not for diagnostic use. The
safety and efficacy of this product in diagnostic or other clinical use has not been established.

For technical questions about the product, call the Life Technologies TECHLINE™ (800) 999-9888.
**Technical Information**

**M0303S**

1,000 units 2,000 U/ml  Lot: 0111303
RECOMBINANT  Store at −20°C  Exp: 3/15

**Description:** DNase I (RNase-free) is an endonuclease that nonspecifically cleaves DNA to release di-, tri- and oligonucleotide products with 5’-phosphorylated and 3’-hydroxylated ends (1,2). DNase I acts on single- and double-stranded DNA, chromatin and RNA:DNA hybrids.

**Source:** An E. coli strain that carries an MBP fusion clone of Bovine Pancreatic DNasel.

**Applications:**
- Degradation of DNA template in transcription reactions
- Removal of contaminating genomic DNA from RNA samples
- DNase I footprinting
- Nick Translation

**Reagents Supplied with Enzyme:**
10X DNase I Reaction Buffer.

**Reaction Conditions:** 1X DNase I Reaction Buffer. Incubate at 37°C.

**1X DNase I Reaction Buffer:**
- 10 mM Tris-HCl
- 2.5 mM MgCl₂
- 0.5 mM CaCl₂
- pH 7.6 @ 25°C

**Unit Definition:** One unit is defined as the amount of enzyme which will completely degrade 1 µg of pBR322 DNA in a total reaction volume of 50 µl in 10 minutes at 37°C.

Complete degradation is defined as the reduction of the majority of DNA fragments to tetranucleotides or smaller.

**Protocol**

**Typical Reaction:**
1. Resuspend 10 µg RNA in 1X DNase I Reaction Buffer to a final volume of 100 µl.
2. Add 2 units of DNase I, mix thoroughly and incubate at 37°C for 10 minutes.
3. Add 1 µl of 0.5 M EDTA (to a final concentration of 5 mM).
4. Heat inactivate at 75°C for 10 minutes.

**Quality Assurance:** Free of Detectable RNases.

**Quality Control Assays**

**RNase Activity:** Incubation of 100 units of DNase I with 10 µg of double-stranded RNA Ladder for 2 hours at 37°C resulted in the same electrophoretic profile as untreated RNA. Incubation of 2 units of DNase I with 10 µg of single-stranded RNA Ladder for 1 hour at 37°C resulted in the same electrophoretic profile as untreated RNA.

**Heat Inactivation:** 75°C for 10 minutes.

**Note:** EDTA should be added to a final concentration of 5 mM to protect RNA from being degraded during enzyme inactivation (3).

**References:**

**Certificate of Analysis**

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159
**T5 Exonuclease**

**M0363S**

<table>
<thead>
<tr>
<th>1,000 units</th>
<th>10,000 U/ml</th>
<th>Lot: 0011307</th>
<th>Exp: 7/15</th>
</tr>
</thead>
</table>

**Description:** T5 Exonuclease degrades DNA in the 5’ to 3’ direction (1). T5 Exonuclease is able to initiate nucleotide removal from the 5’ termini or at gaps and nicks of linear or circular dsDNA (1). However, the enzyme does not degrade supercoiled dsDNA (2). The ssDNA exonuclease activity has been shown to be suppressed by lowering the concentration of Mg<sup>2+</sup> to less than 1 mM (1). This protein is the product of T5 phage D15 gene (3).

**Source:** An *E. coli* strain that carries a plasmid with the T5 phage D15 gene.

Supplied in: 100 mM NaCl, 50 mM Tris-HCl (pH 7.5 @ 25°C), 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100 and 50% glycerol.

**Applications:**

- Degradation of linear ssDNA, dsDNA or nicked plasmid DNA while reserving supercoiled plasmid DNA.

**Reagents Supplied with Enzyme:**

10X NEBuffer 4

**Reaction Conditions:** 1X NEBuffer 4. Incubate at 37°C.

**1X NEBuffer 4:**
50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
1 mM dithiothreitol
pH 7.9 @ 25°C

**Unit Definition:** One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble deoxyribonucleotide from double-stranded DNA in a total reaction volume of 50 μl in 30 minutes at 37°C in 1X NEBuffer 4 with 0.15 mM sonicated duplex [H]-DNA.

**Unit Assay Conditions:** 50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1 mM dithiothreitol (pH 7.9) and 0.15 mM sonicated duplex [H]-DNA.

**Heat Inactivation:** No

**Quality Control Assays**

**Endonuclease Activity:** Endonuclease Activity: Incubation of a 50 μl reaction containing 30 units of T5 Exonuclease with 1 μg of supercoiled pUC19 DNA in NEBuffer 4 for 16 hours at 37°C resulted in < 10% loss in supercoiled pUC19 DNA as determined by agarose gel electrophoresis.

**Physical Purity:** Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

**References:**

Lambda Exonuclease

M0262S

1,000 units 5,000 U/ml Lot: 0071302
RECOMBINANT Store at –20°C Exp: 2/15

Description: A highly processive enzyme that acts in the 5’ to 3’ direction, catalyzing the removal of 5’ mononucleotides from duplex DNA. The preferred substrate is 5’-phosphorylated double stranded DNA, although it will also degrade single-stranded and non-phosphorylated substrates at a greatly reduced rate. Lambda Exonuclease is unable to initiate DNA digestion at nicks or gaps (1).

Source: A genetic fusion of the E. coli Lambda Exonuclease gene with the gene encoding maltose binding protein (MBP). Following affinity chromatography, Lambda Exonuclease is cleaved from the fusion construct and purified away from MBP.

Supplied in: 50 mM NaCl, 25 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 1 mM dithiothreitol and 50% glycerol.

Reagents Supplied with Enzyme: 10X Lambda Exonuclease Reaction Buffer.

Reaction Conditions: 1X Lambda Exonuclease Reaction Buffer. Incubate at 37°C.

1X Lambda Exonuclease Reaction Buffer: 67 mM Glycine-KOH 2.5 mM MgCl₂ 50 μg/ml BSA (pH 9.4 @ 25°C)

Unit Definition: One unit is defined as the amount of enzyme required to produce 10 nmol of acid soluble deoxyribonucleotide from double-stranded substrate in a total reaction volume of 50 μl in 30 minutes at 37°C.

Unit Assay Conditions: 67 mM Glycine-KOH (pH 9.4), 2.5 mM MgCl₂, 50 μg/ml BSA and 1μg sonicated duplex ³²P DNA.

Heat Inactivation: 75°C for 10 minutes.

Quality Control Assays

Endonuclease Activity: Incubation of 200 units of Lambda Exonuclease with 1 μg αX174 RF I DNA for 4 hours at 37°C in 50 μl reaction buffer resulted in < 10% conversion to RF II.

Quality Assurance: Purified free of contaminating endonucleases and exonucleases.

Note: 5’-OH ends are digested 20X slower than 5’-PO₄ ends. Single-strand is digested 100X slower than double-stranded DNA (1).

Reference:
Appendix 10

Mung Bean Nuclease

M0250S

1,500 units Lot: 0251305 Exp: 5/15
10,000 U/ml Store at −20°C

Description: A single-strand specific DNA and RNA endonuclease which will degrade single-stranded extensions from the ends of DNA and RNA molecules, leaving blunt, ligatable ends.

Source: Mung bean sprouts

Molecular Weight: 39 kDa

Supplied in: 10 mM sodium acetate (pH 5.0) 0.1 mM zinc acetate, 1 mM cysteine, 0.001% Triton X-100 and 50% glycerol.

Applications:
- Removal of 3' and 5' extensions from DNA or RNA termini
- Transcriptional mapping
- Cleavage of hairpin loops
- Excision of gene coding sequences from genomic DNA
- Generation of new restriction sites

Note: It is no longer necessary to supplement Mung Bean Nuclease reactions with Zn²⁺. The zinc acetate in the storage buffer fulfills the Zn²⁺ requirement of the enzyme even after dilution in a reaction.

Reagents Supplied with Enzyme:
- 1X Mung Bean Nuclease Reaction Buffer

Reaction Conditions: Substrate DNA at a concentration of 0.1 µg/µl in 1X Mung Bean Nuclease Reaction Buffer. Incubate at 30°C.

1X Mung Bean Nuclease Reaction Buffer:
- 50 mM sodium acetate
- 30 mM NaCl
- 1 mM ZnSO₄
- pH 5.0 @ 25°C

Also active in NEBuffers 1, 2 & 4.

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 µg of acid-soluble total nucleotide in 1 minute at 37°C.

Unit Assay Conditions: 1X Mung Bean Nuclease Reaction Buffer and 0.5 mg/ml denatured calf thymus DNA as an enzyme substrate.

Removal of Single-Stranded Extensions:
1. Suspend DNA (0.1 µg/µl) in 1X Mung Bean Nuclease Reaction Buffer or 1X NEBuffers 1, 2, or 4.
2. Add 1.0 unit of Mung Bean Nuclease per µg DNA.
3. Incubate at 30°C for 30 minutes.
4. Inactivate the enzyme by phenol/chloroform extraction or by addition of SDS to 0.01%.
5. Recover the DNA by ethanol precipitation.

Quality Assurance: Purified free of double-strand exonuclease contamination.

Quality Control Assays
16 µg of Hae III digested qX174 DNA was incubated with 10 units of Mung Bean Nuclease in a 400 µl volume of 1X NEBuffer 2 for 30 minutes at 30°C. The DNA was then precipitated, ligated with T4 DNA Ligase and recut. 90% of the DNA fragments treated with Mung Bean Nuclease were ligated and of those 95% were recut with Hae III.

References:

Certificate of Analysis

162
Lambda DNA

N3011L

1,250 µg  Lot: 1641308  Exp: 8/15
500 µg/ml  Store at -20°C

Description: Duplex DNA is isolated from bacteriophage lambda (cl857 ind 1 Sam 7). Lambda DNA is 48,502 base pairs in length.

Source: The phage is isolated from the heat-inducible lysogen E. coli λ cl857 S7. The DNA is isolated from the purified phage by phenol extraction and dialyzed against 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA.

Supplied in: 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA.

Molecular Weight: 31.5 x 10^6 daltons

Reference:
Appendix 10

DNA/Hind III Fragments

Cat. No. 15612-013

Conc.: 8.5 μg/μl

Size: 500 μg

Store at -20°C.

Description:

λ DNA/Hind III Fragments are prepared from λ DNA (clind1nt857 Sam7) that has been digested to completion with Hind III. They are suitable for sizing linear double-stranded DNA from 125 bp to 23.1 kb. These fragments can be visualized by ethidium bromide staining. The fragments can also be radioactively labeled by filling in the 3' recessed ends with E. coli DNA polymerase I or the large fragment of DNA polymerase I.

Storage Buffer:
10 mM Tris-HCl (pH 7.4)
5 mM NaCl
0.1 mM EDTA

Recommended Procedure:

Invitrogen recommends the use of 10X BluGelR™ Gel Loading Buffer (10816015) at a final concentration of 2X (for electrophoresis of this marker on agarose gels). Alternatively, this DNA marker can be diluted in a buffer such that the final concentration of NaCl is 20 mM. Apply approximately 0.1 μg of standard per mm lane width. Heat to 65°C for no longer than 10 minutes immediately prior to loading. Failure to heat before use will cause the 4361 bp band to be faint or absent.

Quality Control:

Presence of the upper six DNA fragments are verified by agarose gel electrophoresis. A260/A280 ratio is in the range of 1.8-2.0.

Reference:


Large Fragment Labeling Protocol:

1. To a 1.5-ml microcentrifuge tube on ice, add the following:
   
<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoclaved water</td>
<td>10 μl</td>
</tr>
<tr>
<td>10X Large Fragment reaction buffer [50 mM Tris-HCl (pH 7.5), 60 mM MgCl₂, 50 mM NaCl]</td>
<td>2 μl</td>
</tr>
<tr>
<td>0.5 mM dATP</td>
<td>1 μl</td>
</tr>
<tr>
<td>0.5 mM dCTP</td>
<td>1 μl</td>
</tr>
<tr>
<td>0.5 mM dGTP</td>
<td>1 μl</td>
</tr>
<tr>
<td>0.5 mM dTTP</td>
<td>1 μl</td>
</tr>
<tr>
<td>λ DNA/Hind III Fragments (0.5 μg)</td>
<td>1 μl</td>
</tr>
<tr>
<td>(α-³²P)ATP (400 Ci/mmol, 10 μC/ml)</td>
<td>2 μl</td>
</tr>
<tr>
<td>Large Fragment (0.5 μl)</td>
<td>1 μl</td>
</tr>
</tbody>
</table>

2. A common restriction enzyme buffer can be substituted for this
   [e.g. 6 mM Tris-HCl (pH 7.5), 6 mM MgCl₂, 1 mM DTT, and 0-50 mM NaCl].

3. Make sure all components are at the bottom of the tube. Mix thoroughly but not vigorously. Centrifuge briefly.

4. Add 5 μl 0.1% (w/v) bromphenol blue, 0.1 mM EDTA, 50% (v/v) glycerol to the sample.

5. Load 1 × 10⁵ cpm in a lane.
1 Kb Plus DNA Ladder

Cat. No. 10787-018

Size: 250 μg

Con.: 1 μg/μl

Store at -20°C.

Description:
The 1 Kb Plus DNA Ladder is suitable for sizing linear double-stranded DNA fragments from 100 bp to 12 kb. The ladder contains a total of twenty bands: twelve bands ranging in size from 1000 bp to 12 000 bp in 1000-bp increments and eight bands ranging in size from 100 to 1650 bp. The 1650-bp band contains approximately 8% of the mass applied to the gel. The ladder may be radioactively labeled by one of the following methods: (i) Partial exonuclease degradation and reassembly with T4 DNA polymerase. This method is preferred because higher specific activity is achieved with less 32P input; (ii) Labelling the 5' ends with T4 polynucleotide kinase; (iii) Filling in the 3' recessed ends with E. coli DNA polymerase I or the large fragment of DNA polymerase 1.

Storage Buffer:

10 mM Tris-HCl (pH 7.5) 1 mM EDTA 50 mM NaCl

Recommended Procedure:

Invitrogen recommends the use of 10X BlueAlu™ Gel Loading Buffer (Cat. No. 10816-015) at a concentration of 2X for electrophoresis of DNA standards on agarose gels. Alternately, the DNA standard can be diluted such that the final concentration of NaCl is 20 mM. Apply approximately 0.1 μg of ladder per mm lane width. Do not heat before loading.

Quality Control:

Agarose gel analysis shows that all bands in the ladder are distinguishable and are of approximate equal intensity by ethidium bromide staining.

Structure of Fragments in 1-Kb Increments:

GATCC — G — CTAG-5' (96% b/a)

Notes:

During 1% agarose gel electrophoresis with Tris-acetate (pH 7.5) as the running buffer, bromophenol blue migrates together with the 500-bp band.

The 1650-bp band is generated from pUC. The bands smaller than 1000 bp are derived from lambda DNA.

Labeling Protocols:

32P DNA Polymerase Labeling Protocol

1. Enzyme Reaction (Degradation of DNA from both 3'-ends)

   a. To a 1.5-μl microcentrifuge tube on ice, add the following:
   - 5X T4 DNA polymerase reaction buffer [165 mM Tris acetate (pH 7.9), 330 mM sodium acetate, 50 mM magnesium acetate, 2.5 mM DTT, 50 μg/ml BSA] 10 μl
   - 1 Kb Plus DNA Ladder 4 μl
   - T4 DNA polymerase 40 units
   - Autoclaved water 20 μl

   b. Make sure all components are at the bottom of the tube. Mix thoroughly but not vigorously. Centrifuge briefly.
   c. Incubate 2 min at 37°C. (about 25 nucleiolids/min are removed).
   d. Cool reaction vial on ice.
   e. Reassembly Reaction (Fill-in)

      This reaction will reassemble the degraded DNA strands.

      a. Place into the reaction vial which is sitting in ice after the exoneuclease reaction:
         - Autoclaved water 8 μl
         - 5X T4 DNA polymerase reaction buffer 6 μl
         - 4CIP (2 mM) 5 μl
         - 4GTP (2 mM) 5 μl
         - DTT (2 mM) 5 μl
         - [γ-32P]ATP (3000 Ci/mmol, 10 μCi/ml) 1 μl

      b. Mix thoroughly. Centrifuge briefly. Incubate 2 min at 37°C, then add 5 μl of 2 mM dATP.
      c. Incubate 2 min at 37°C. Stop reaction by adding 2.5 μl of 0.5 M EDTA. Centrifuge for 10 s.
      d. The cpm incorporated is determined by adding 1 μl of reaction to 24 μl of 250 mM NaCl, 25 mM EDTA. Spot 5 μl of dilution on a glass filter paper. Place filter in 10% (w/v) TCA + 1% (w/v) pyrophosphate. Wash filter 3 times with 3% (w/v) TCA and then 2 times with ethanol. The filter is dried and then counted using an appropriate scintillant.

 2. DNA Terminus Labeling Protocol (Phosphate Exchange Reaction)

      This reaction will yield specific activities of approximately 1 × 106 cpm/pmol of ends.

      5'- ...32P... -P -P -P -ATP + ADP 11 phosphoenol, 32P-...32P... -ATP + ADP

      1. Add the following components to a 0.5-ml microcentrifuge tube in the following order:
         - Autoclaved water 11 μl
         - 1 Kb Plus DNA Ladder 5 μl
         - 5X exchange reaction buffer [250 mM imidazole (pH 6.4), 1.5 mM ATP, 60 mM MgCl2, 75 mM 2-mercaptoethanol] 5 μl
         - [γ-32P]ATP (10 μCi/μl) 1 μl
         - T4 polynucleotide kinase (5 or 10 U/μl) 1 μl

      2. Incubate the reaction mixture at 37°C for 30 minutes. Increasing reaction times beyond 30 min will not increase labeling of the DNA.
      3. Stop reaction by adding 1 μl of 0.5 M EDTA. Centrifuge for 10 s.
      4. Determine radioactive incorporation as above.
      5. Add 5 μl 0.1% (w/v) bromophenol blue, 0.1 mM EDTA, 50% (w/v) glycerol to the sample.