

5' DNA Adenylation Kit



1-800-632-7799
info@neb.com
www.neb.com



E2610S 003161218121

E2610S



10 reactions Lot: 0031612

RECOMBINANT Store at -20°C Exp: 12/18

Description: The 5' DNA adenylation Kit is a simple and efficient enzymatic method for generating 5'-adenylated DNA. The kit is optimized to produce the adenylated DNA with or without 3'-terminator. The 5' DNA adenylation kit routinely generates greater than 95% conversion of pDNA to AppDNA (1). This highly efficient process eliminates the need for gel isolation of the product and increases overall yield.

Source: *Mth* RNA Ligase is purified from an *E. coli* strain carrying a plasmid encoding thermostable RNA ligase from *Methanobacterium thermoautotrophicum* (2).

Advantages:

- One step reaction gives quantitative adenylation. Simpler than existing chemical and enzymatic methods.
- Reduces need for extensive purification of reaction product.
- 65°C reaction temperature reduces secondary structural concerns.
- Easily scalable from pmol to μmol range.

Application:

- Enzymatic 5'-adenylation of single-stranded DNA linkers for next generation sequencing.

Kit Components:

Mth RNA Ligase
10X 5' DNA Adenylation Reaction Buffer
1 mM ATP

Supplied in: *Mth* RNA Ligase is supplied in 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 1 mM DTT, 0.1 mM EDTA and 50% glycerol.

1X 5' DNA Adenylation Reaction Buffer:

50 mM Sodium Acetate (pH 6.0 @ 25°C)
10 mM MgCl₂
5 mM DTT
0.1 mM EDTA
Supplement with 0.1 mM ATP, Incubate at 65°C

Protocol for Oligonucleotide Adenylation:

1. Set up the following reaction in a sterile microfuge tube:

COMPONENTS	VOLUME
Phosphorylated DNA Oligonucleotide	100 pmol (5 pmol/μl)
10X 5' DNA Adenylation Reaction Buffer	2 μl
1 mM ATP	2 μl
<i>Mth</i> RNA Ligase	2 μl (100 pmol)
Nuclease-free Water	to 20 μl

2. Incubate at 65°C for 1 hour
3. Inactivate the enzyme by incubation at 85°C for 5 minutes

Quality Control Assays

RNase Assay: A 10 μl reaction in 5' DNA Adenylation Reaction Buffer containing 40 ng of labeled RNA and 100 pmol of *Mth* RNA Ligase incubated at 37°C. After incubation for 4 hours, > 90% of the substrate RNA remains intact as determined by polyacrylamide electrophoresis.

Exonuclease Activity: Incubation of a 50 μl reaction containing 100 pmol of *Mth* RNA Ligase with 1 μg of a mixture of single and double-stranded ³H *E. coli* DNA (200,000 cpm/μg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of a 50 μl reaction containing 100 pmol of *Mth* RNA Ligase with 1 μg φX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RFI as determined by agarose gel electrophoresis.

Phosphatase Activity: Incubation of 100 pmol of *Mth* RNA Ligase with 2.5 μmol *p*-nitrophenyl phosphate (PNPP) in 50 μl Reaction Buffer for 3 hours at 65°C released less than 0.05 μmol inorganic phosphate.

(see other side)

CERTIFICATE OF ANALYSIS

5' DNA Adenylation Kit



1-800-632-7799
info@neb.com
www.neb.com



E2610S 003161218121

E2610S



10 reactions Lot: 0031612

RECOMBINANT Store at -20°C Exp: 12/18

Description: The 5' DNA adenylation Kit is a simple and efficient enzymatic method for generating 5'-adenylated DNA. The kit is optimized to produce the adenylated DNA with or without 3'-terminator. The 5' DNA adenylation kit routinely generates greater than 95% conversion of pDNA to AppDNA (1). This highly efficient process eliminates the need for gel isolation of the product and increases overall yield.

Source: *Mth* RNA Ligase is purified from an *E. coli* strain carrying a plasmid encoding thermostable RNA ligase from *Methanobacterium thermoautotrophicum* (2).

Advantages:

- One step reaction gives quantitative adenylation. Simpler than existing chemical and enzymatic methods.
- Reduces need for extensive purification of reaction product.
- 65°C reaction temperature reduces secondary structural concerns.
- Easily scalable from pmol to μmol range.

Application:

- Enzymatic 5'-adenylation of single-stranded DNA linkers for next generation sequencing.

Kit Components:

Mth RNA Ligase
10X 5' DNA Adenylation Reaction Buffer
1 mM ATP

Supplied in: *Mth* RNA Ligase is supplied in 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 1 mM DTT, 0.1 mM EDTA and 50% glycerol.

1X 5' DNA Adenylation Reaction Buffer:

50 mM Sodium Acetate (pH 6.0 @ 25°C)
10 mM MgCl₂
5 mM DTT
0.1 mM EDTA
Supplement with 0.1 mM ATP, Incubate at 65°C

Protocol for Oligonucleotide Adenylation:

1. Set up the following reaction in a sterile microfuge tube:

COMPONENTS	VOLUME
Phosphorylated DNA Oligonucleotide	100 pmol (5 pmol/μl)
10X 5' DNA Adenylation Reaction Buffer	2 μl
1 mM ATP	2 μl
<i>Mth</i> RNA Ligase	2 μl (100 pmol)
Nuclease-free Water	to 20 μl

2. Incubate at 65°C for 1 hour
3. Inactivate the enzyme by incubation at 85°C for 5 minutes

Quality Control Assays

RNase Assay: A 10 μl reaction in 5' DNA Adenylation Reaction Buffer containing 40 ng of labeled RNA and 100 pmol of *Mth* RNA Ligase incubated at 37°C. After incubation for 4 hours, > 90% of the substrate RNA remains intact as determined by polyacrylamide electrophoresis.

Exonuclease Activity: Incubation of a 50 μl reaction containing 100 pmol of *Mth* RNA Ligase with 1 μg of a mixture of single and double-stranded ³H *E. coli* DNA (200,000 cpm/μg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of a 50 μl reaction containing 100 pmol of *Mth* RNA Ligase with 1 μg φX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RFI as determined by agarose gel electrophoresis.

Phosphatase Activity: Incubation of 100 pmol of *Mth* RNA Ligase with 2.5 μmol *p*-nitrophenyl phosphate (PNPP) in 50 μl Reaction Buffer for 3 hours at 65°C released less than 0.05 μmol inorganic phosphate.

(see other side)

CERTIFICATE OF ANALYSIS

Usage Notes:

- The adenylation reaction can be scaled up to 6X without a loss of efficiency, to a final concentration of 30 pmol of oligonucleotide and 30 pmol of *Mth* RNA Ligase per μ l. The oligonucleotide can be purified by phenol extraction and alcohol precipitation or column chromatography to remove protein and ATP.
- For substrates with unprotected 3' termini increase concentration of ATP to 0.5 mM to prevent circularization and concatemerization.
- The low turnover of the enzyme requires an approximately equimolar concentration of the enzyme and the oligonucleotide substrate.
- Adenylated DNA linkers can be used for 3'-end ligation of RNA in cDNA library preparation for Next Generation sequencing protocols [3,4].

References:

1. Zhelkovsky, A.M. and McReynolds, L.A. (2011) *Nucl. Acids Res.* 39(17): e117.
2. Torchia, C., Takagi, Y. and Ho, C.K. (2008) *Nucleic Acids Res.*, 36, 6218–6227.
3. Hafner, M. et al. (2008) *Methods*, 44, 3–12.
4. Vigneault, F., Sismour, A.M. and Church, G.M. (2008) *Nature Methods*, 5, 777–779.



NEW ENGLAND BIOLABS® is a registered trademark of New England Biolabs, Inc.

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Usage Notes:

- The adenylation reaction can be scaled up to 6X without a loss of efficiency, to a final concentration of 30 pmol of oligonucleotide and 30 pmol of *Mth* RNA Ligase per μ l. The oligonucleotide can be purified by phenol extraction and alcohol precipitation or column chromatography to remove protein and ATP.
- For substrates with unprotected 3' termini increase concentration of ATP to 0.5 mM to prevent circularization and concatemerization.
- The low turnover of the enzyme requires an approximately equimolar concentration of the enzyme and the oligonucleotide substrate.
- Adenylated DNA linkers can be used for 3'-end ligation of RNA in cDNA library preparation for Next Generation sequencing protocols [3,4].

References:

1. Zhelkovsky, A.M. and McReynolds, L.A. (2011) *Nucl. Acids Res.* 39(17): e117.
2. Torchia, C., Takagi, Y. and Ho, C.K. (2008) *Nucleic Acids Res.*, 36, 6218–6227.
3. Hafner, M. et al. (2008) *Methods*, 44, 3–12.
4. Vigneault, F., Sismour, A.M. and Church, G.M. (2008) *Nature Methods*, 5, 777–779.



NEW ENGLAND BIOLABS® is a registered trademark of New England Biolabs, Inc.

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.