5´ DNA Adenylation Kit

1-800-632-7799
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E2610S 001121214121

E2610S

RR

10 reactions **Lot: 0011212**

RECOMBINANT **Store at –20°C** **Exp: 12/14**

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 $MgCl_2$
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
Mth 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 RFII 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

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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 µl. 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.

Patent Pending

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