**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.

**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

**Protocol for Oligonucleotide Adenylation:**
1. Set up the following reaction in a sterile microfuge tube:

<table>
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<tr>
<th>COMPONENTS</th>
<th>VOLUME</th>
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<tbody>
<tr>
<td>Phosphorylated DNA</td>
<td>100 pmol (5 pmol/µl)</td>
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<tr>
<td>Oligonucleotide</td>
<td>2 µl</td>
</tr>
<tr>
<td>10X 5’ DNA Adenylation</td>
<td>1 mM ATP</td>
</tr>
<tr>
<td>Reaction Buffer</td>
<td>2 µl</td>
</tr>
<tr>
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<tr>
<td>Nuclease-free Water</td>
<td>to 20 µl</td>
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2. Incubate at 65°C for 1 hour
3. Inactivate the enzyme by incubation at 85°C for 5 minutes

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

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**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.

**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 *E. coli* DNA (200,000 cpm/µg) for 4 hours at 37°C resulted in <0.1% of the total radioactivity.

**Endonuclease Activity:** Incubation of a 50 µl reaction containing 100 pmol of Mth RNA Ligase with 1 µg of 174 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)
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:

Patent Pending