The 5’ DNA Adenylation Kit is a simple and efficient enzymatic method for generating 5’-adenylated DNA. The kit is optimized to produce the adénylated 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

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

**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 <10% conversion to RFII as determined by agarose gel electrophoresis.
- **Phosphatase Activity:** Incubation of 100 pmol of *Mth* RNA Ligase with 2.5 µmol 5-nitrophenyl phosphate (PNPP) in 50 µl Reaction Buffer for 3 hours at 65°C released less than 0.05 µmol inorganic phosphate.

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

<table>
<thead>
<tr>
<th>COMPONENTS</th>
<th>VOLUME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorylated DNA</td>
<td>100 pmol (5 pmol/µl)</td>
</tr>
<tr>
<td>Oligonucleotide</td>
<td>2 µl</td>
</tr>
<tr>
<td>10X 5’ DNA Adenylation Reaction Buffer</td>
<td>2 µl</td>
</tr>
<tr>
<td>1 mM ATP</td>
<td>2 µl</td>
</tr>
<tr>
<td>ATP</td>
<td>2 µl</td>
</tr>
<tr>
<td>Nuclease-free Water</td>
<td>to 20 µl</td>
</tr>
</tbody>
</table>

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