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:

<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>1 µl</td>
</tr>
<tr>
<td>10X 5’ DNA Adenylation Reaction Buffer</td>
<td>0.5 µl</td>
</tr>
<tr>
<td>1 mM ATP</td>
<td>2 µl</td>
</tr>
<tr>
<td>ATP RNA Ligase</td>
<td>2 µl (100 pmol)</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

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 α-H. E. coli DNA (2,000,000 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 of a mixture of single and double-stranded α-H. E. coli DNA (2,000,000 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 p-nitrophenyl phosphate (PNPP) in 50 µl Reaction Buffer for 3 hours at 65°C released less than 0.05 µmol inorganic phosphate.

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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