Thermostable 5’ App DNA/RNA Ligase

10 reactions 20 µM Lot: 0011409
RECOMBINANT Store at –20°C Exp: 9/16

Description: Thermostable 5’ App DNA/RNA Ligase is a point mutant of catalytic lysine of RNA ligase from *Methanobacterium thermoautotrophicum* (1). This enzyme is ATP independent. It requires a 5’-adenylated linker for ligation to the 3’-OH end of either RNA or single stranded DNA (ssDNA). The enzyme is also active in ligation of RNA with 2’-O-methylated 3’-end to 5’-adenylated linkers (1). The optimal temperature for ligation reaction is 60–65°C (2). The mutant ligase is unable to adenylate the 5’-phosphate of RNA or ssDNA, which reduces the formation of undesired ligation products (concatemers and circles). The ability of the ligase to function at 65°C might reduce the constraints of RNA secondary structure in RNA ligation experiments.

Source: Thermostable 5’ App DNA/RNA Ligase is expressed as His-tag fusion in *E. coli*.

Applications:
- Ligate a 5’-adenylated 3’-blocked DNA or RNA sequence tag to the 3’-OH of RNA and ssDNA.

Reagents Supplied with Enzyme:
- NEBuffer 1 (10X) and 0.1 mM EDTA, 1 mM DTT and 50% glycerol.

Reaction Conditions: 1X NEBuffer 1 (supplemented with 5 mM MnCl₂ for ssDNA substrate only). Incubate at 65°C.

Notes on Use: For optimal ligation of ssDNA to adenylated DNA linkers, we recommend using NEBuffer 1 supplemented with manganese. For ligation of ssRNA to adenylated DNA linkers, just use NEBuffer 1. Heat inactivation or Proteinase K treatment is required to release RNA bound by the ligase.

1X NEBuffer 1:
- 10 mM Bis-Tris-Propane-HCl
- 10 mM MgCl₂
- 1 mM Dithiothreitol
- pH 7.0 @ 25°C

Protocol for ssDNA/RNA Ligation:
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>ssDNA/RNA Substrate</td>
<td>20 pmol (1 pmol/µl)</td>
</tr>
<tr>
<td>5 App DNA Digoxigencode</td>
<td>40 pmol (2 pmol/µl)</td>
</tr>
<tr>
<td>10X NEBuffer 1</td>
<td>2 µl</td>
</tr>
<tr>
<td>50 mM MnCl₂ (for ssDNA ligation only)</td>
<td>2 µl</td>
</tr>
<tr>
<td>Thermostable 5’ App DNA/RNA Ligase</td>
<td>2 µl (40 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 90°C for 3 minutes.

Quality Control Assays

RNase Assay: A 10 µl reaction in 1X NEBuffer 1 containing 40 ng of labeled RNA and 100 pmol of Thermostable 5’ App DNA/RNA Ligase incubated at 37°C. After incubation for 16 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 Thermostable 5’ App DNA/RNA Ligase with 1 µg of a mixture of single and double-stranded *H. coli* DNA (200,000 cpm/µg) for 4 hours at 37°C released < 10% conversion to RF II as determined by agarose gel electrophoresis.

(see other side)
Phosphatase Activity: Incubation of 100 pmol of Thermostable 5´ App DNA/RNA Ligase with 2.5 µmol p-nitrophenyl phosphate (PNPP) in 50 µl Reaction Buffer for 16 hours at 37°C released less than 0.05 µmol inorganic phosphate

References:

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