Product Name: 5’ DNA Adenylation Kit  
Catalog Number: E2610S  
Concentration: 50 µM  
Lot Number: 10009214  
Expiration Date: 04/2020  
Storage Temperature: -20°C  
Specification Version: PS-E2610S/L v1.0

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**5’ DNA Adenylation Kit Component List**

<table>
<thead>
<tr>
<th>NEB Part Number</th>
<th>Component Description</th>
<th>Lot Number</th>
<th>Individual QC Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>N0757AVIAL</td>
<td>Adenosine 5’ Triphosphate</td>
<td>0121804</td>
<td>Pass</td>
</tr>
<tr>
<td>M2611AVIAL</td>
<td>Mth RNA Ligase</td>
<td>0031804</td>
<td>Pass</td>
</tr>
<tr>
<td>B2610SVIAL</td>
<td>5’ DNA Adenylation Reaction Buffer</td>
<td>10008138</td>
<td>Pass</td>
</tr>
</tbody>
</table>

**Assay Name/Specification**

**Protein Purity Assay (SDS-PAGE)**

Mth RNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

**Phosphatase Activity (pNPP)**

A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl2 containing 2.5 mM p-Nitrophenyl Phosphate (pNPP) and a minimum of 50 pmol of Mth RNA Ligase incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.

**RNase Activity Assay (4 Hour Digestion)**

A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 50 pmol of Mth RNA Ligase is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

*Individual Product Component Note*

Standard Quality Control Tests are performed for each component included in 5’ DNA Adenylation Kit and meet the designated specifications.

**Endonuclease Activity (Nicking)**

A 50 µl reaction in 1X 5’ DNA Adenylation Reaction Buffer containing 1 µg of
<table>
<thead>
<tr>
<th>Assay Name/Specification</th>
<th>Lot # 10009214</th>
</tr>
</thead>
<tbody>
<tr>
<td>supercoiled PhiX174 DNA and a minimum of 50 pmol of Mth RNA Ligase incubated for 4 hours at 37°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</td>
<td></td>
</tr>
<tr>
<td><strong>Exonuclease Activity (Radioactivity Release)</strong></td>
<td>Pass</td>
</tr>
<tr>
<td>A 50 µl reaction in 1X 5’ DNA Adenylation Reaction Buffer containing 1 µg of a mixture of single and double-stranded [3H] E. coli DNA and a minimum of 50 pmol of Mth RNA Ligase incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</td>
<td></td>
</tr>
<tr>
<td><strong>Functional Testing (Adenylation)</strong></td>
<td>Pass</td>
</tr>
<tr>
<td>A 20 µl reaction in 1X 5’ DNA Adenylation Reaction Buffer containing 100 pmol of 17 bp long 5’P-ssDNA linker (5’-pCTGTAGGCACCATCAAT–NH2-3’), 0.1 mM ATP, and 100 pmol of Mth RNA Ligase incubated for 1 hour at 65°C results in ≥95% 5’P-ssDNA linker adenylated.</td>
<td></td>
</tr>
</tbody>
</table>

This product has been tested and shown to be in compliance with all specifications.

________________________________________
Bhairavi Jani  
Production Scientist  
15 Jun 2018

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Mary Conlon  
Packaging Quality Control Inspector  
15 Jun 2018