

## New England Biolabs Certificate of Analysis

Product Name: 5' DNA Adenylation Kit  
 Catalog Number: E2610L  
 Concentration: 50  $\mu$ M  
 Packaging Lot Number: 10091344  
 Expiration Date: 11/2022  
 Storage Temperature: -20°C  
 Specification Version: PS-E2610S/L v1.0

5' DNA Adenylation Kit Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
N0757AVIAL	Adenosine 5' Triphosphate	10089439	Pass
M2611AAVIAL	Mth RNA Ligase	10089437	Pass
B2610SVIAL	5' DNA Adenylation Reaction Buffer	10068005	Pass

Assay Name/Specification	Lot # 10091344
<p><b>* Individual Product Component Note</b>            Standard Quality Control Tests are performed for each component included in 5' DNA Adenylation Kit and meet the designated specifications.</p>	Pass
<p><b>RNase Activity Assay (4 Hour Digestion)</b>            A 10 <math>\mu</math>l 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, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>	Pass
<p><b>Protein Purity Assay (SDS-PAGE)</b>            Mth RNA Ligase is <math>\geq</math> 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	Pass
<p><b>Phosphatase Activity (pNPP)</b>            A 200 <math>\mu</math>l reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl<sub>2</sub> 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 &lt;0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.</p>	Pass
<p><b>Functional Testing (Adenylation)</b>            A 20 <math>\mu</math>l reaction in 1X 5' DNA Adenylation Reaction Buffer containing 100 pmol of 17</p>	Pass

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<p>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.</p>	
<p><b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in 1X 5' DNA Adenylation Reaction Buffer containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] 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.</p>	<b>Pass</b>
<p><b>Endonuclease Activity (Nicking)</b> A 50 µl reaction in 1X 5' DNA Adenylation Reaction Buffer containing 1 µg of 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.</p>	<b>Pass</b>

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

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