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240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## New England Biolabs Certificate of Analysis

Product Name:	5' DNA Adenylation Kit
Catalog Number:	E2610S
Concentration:	50 µM
Packaging Lot Number:	10136950
Expiration Date:	10/2023
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	10125488	Pass	
M2611AVIAL	Mth RNA Ligase	10125490	Pass	
B2610SVIAL	5' DNA Adenylation Reaction Buffer	10105412	Pass	

Assay Name/Specification	Lot # 10136950
<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 <0.1% of the total radioactivity.	Pass
Functional Testing (Adenylation) 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.	Pass
<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 <10% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
<b>RNase Activity Assay (4 Hour Digestion)</b> A 10 μI 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	Pass





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for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.	
<b>Protein Purity Assay (SDS-PAGE)</b> Mth RNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
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.	Pass
* Individual Product Component Note Standard Quality Control Tests are performed for each component included in 5' DNA Adenylation Kit and meet the designated specifications.	Pass

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

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Bhairavi Jani Production Scientist 16 Feb 2022

Josh Hersey

Packaging Quality Control Inspector 16 Feb 2022

