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## New England Biolabs Certificate of Analysis

Product Name: 5' DNA Adenylation Kit

Catalog Number: E2610L
Concentration: 50 µM
Lot Number: 10040004
Expiration Date: 11/2020
Storage Temperature: -20°C

Specification Version: PS-E2610S/L v1.0

5' DNA Adenylation Kit Component List				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
N0757AVIAL	Adenosine 5' Triphosphate	10028386	Pass	
M2611AAVIAL	Mth RNA Ligase	10028383	Pass	
B2610SVIAL	5' DNA Adenylation Reaction Buffer	10008138	Pass	

Assay Name/Specification	Lot # 10040004
Endonuclease Activity (Nicking) 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
Exonuclease Activity (Radioactivity Release) 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 <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
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	Pass



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Assay Name/Specification	Lot # 10040004
for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.	
Protein Purity Assay (SDS-PAGE)  Mth RNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
RNase Activity Assay (4 Hour Digestion) A 10 $\mu$ I 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.	Pass
* Individual Product Component Note Standard Quality Control Tests are performed for each component included in 5' DNA	Pass

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

Adenylation Kit and meet the designated specifications.

Bo Wu `

Production Scientist

03 Dec 2018

Michael Tonello

Packaging Quality Control Inspector

15 Apr 2019



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