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## New England Biolabs Certificate of Analysis

Product Name: 5' DNA Adenylation Kit

Catalog Number:E2610SConcentration: $50 \mu M$ Packaging Lot Number:10176041Expiration Date:10/2023Storage Temperature: $-20^{\circ}$ 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	10172045	Pass	
M2611AVIAL	Mth RNA Ligase	10125490	Pass	
B2610SVIAL	5' DNA Adenylation Reaction Buffer	10125489	Pass	

Assay Name/Specification	Lot # 10176041
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
Protein Purity Assay (SDS-PAGE)  Mth RNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	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
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.	Pass
Endonuclease Activity (Nicking) A 50 μl reaction in 1X 5' DNA Adenylation Reaction Buffer containing 1 μg of	Pass



E2610S / Lot: 10176041

Page 1 of 2

Assay Name/Specification	Lot # 10176041
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.	
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
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

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

Production Scientist 13 Oct 2021 Michael Tonello

Packaging Quality Control Inspector

29 Dec 2022



E2610S / Lot: 10176041

Page 2 of 2