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## New England Biolabs Certificate of Analysis

Product Name: 5' DNA Adenylation Kit

Catalog Number: E2610S
Concentration: 50 µM
Lot Number: 10053874
Expiration Date: 07/2021
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	10049239	Pass	
M2611AVIAL	Mth RNA Ligase	10049238	Pass	
B2610SVIAL	5' DNA Adenylation Reaction Buffer	10042923	Pass	

Assay Name/Specification	Lot # 10053874
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
* 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
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



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Assay Name/Specification	Lot # 10053874
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 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

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

Bo Wu

Production Scientist

11 Jul 2019

Josh Hersey

Packaging Quality Control Inspector

12 Sep 2019



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