

New England Biolabs Product Specification

<i>Product Name:</i>	<i>5' DNA Adenylation Kit</i>
<i>Catalog #:</i>	<i>E2610S/L</i>
<i>Kit Components:</i>	<i>Mth RNA Ligase (M2611)</i> <i>5' DNA Adenylation Reaction Buffer (B2610)</i> <i>Adenosine 5' Triphosphate (N0757)</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Specification Version:</i>	<i>PS-E2610S/L v1.0</i>
<i>Effective Date:</i>	<i>03 Apr 2018</i>

Assay Name/Specification (minimum release criteria)

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.

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.

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.

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.

Protein Purity Assay (SDS-PAGE) - Mth RNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

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.



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* Individual Product Component Note - Standard Quality Control Tests are performed for each component included in 5' DNA Adenylation Kit and meet the designated specifications.
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Date 03 Apr 2018

Derek Robinson
Director of Quality Control

