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New England Biolabs Certificate of Analysis

| Product Name: | E. coli Poly(A) Polymerase |
|------------------------|--|
| Catalog #: | M0276S/L |
| Concentration: | 5,000 units/ml |
| Unit Definition: | One unit is defined as the amount of enzyme that will incorporate 1 nmol of AMP into RNA in a 20 μ l volume in 10 minutes at 37°C. |
| Lot #: | 0171804 |
| Assay Date: | 04/2018 |
| Expiration Date: | 04/2020 |
| Storage Temp: | -20°C |
| Storage Conditions: | 20 mM Tris-HCl, 300 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.1 % Triton®X-100, 50% Glycerol, (pH 7.5 @ 25°C) |
| Specification Version: | PS-M0276S/L v1.0 |
| Effective Date: | 07 May 2018 |

| Assay Name/Specification (minimum release criteria) | |
|---|------|
| Endonuclease Activity (Nicking) - A 50 μ l reaction in Poly(A) Polymerase Reaction Buffer containing 1 μ g of supercoiled PhiX174 DNA and a minimum of 15 units of <i>E. coli</i> Poly(A) Polymerase 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 μ l reaction in Poly(A) Polymerase Reaction Buffer containing 1 μ g of a mixture of single and double-stranded [³ H] <i>E. coli</i> DNA and a minimum of 15 units of <i>E. coli</i> Poly(A) Polymerase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. | Pass |
| Protein Purity Assay (SDS-PAGE) - <i>E. coli</i> Poly(A) Polymerase is \ge 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | Pass |
| RNase Activity (Extended Digestion) - A 10 μ l reaction in NEBuffer 4 containing 40 ng of a 300 base single- stranded RNA and a minimum of 5 units of <i>E. coli</i> Poly(A) Polymerase 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 |

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Authorized by Derek Robinson 07 May 2018



Inspected by Bhairavi Jani 03 Apr 2018