

New England Biolabs Certificate of Analysis

Product Name: RecA
Catalog #: M0249S/L
Concentration: 2 mg/ml
Lot #: 0291802
Assay Date: 02/2018
Expiration Date: 02/2020
Storage Temp: -20°C
Storage Conditions: 10 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, (pH 7.4 @ 25°C)
Specification Version: PS-M0249S/L v1.0
Effective Date: 27 Apr 2018

Assay Name/Specification (minimum release criteria)	Lot #0291802
<p>Endonuclease Activity (Nicking) - A 50 µl reaction in RecA Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 10 µg of RecA incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	Pass
<p>Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in RecA Reaction Buffer containing 1 µg of a mixture of single and double-stranded [³H] <i>E. coli</i> DNA and a minimum of 10 µg of RecA incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.</p>	Pass
<p>Functional Testing (Triple Helix Formation) - The plasmid pUC19 contains 5 HpyCH4IV sites. A 60-mer was designed with complementarity to the region centered around the HpyCH4IV site at position 374. A reaction containing 1 µg pUC19, 0.18 µg 60-mer, 0.3 mM ATP γ-S, 4 µg RecA, in 40 µl 1X RecA Reaction Buffer was incubated at 37°C for 10 minutes to form a stable triple helix. The unprotected sites were methylated using 8 units of SssI supplemented with 160 µM SAM for 10 minutes at 37°C. The reaction was stopped and the triple helix disrupted by incubation at 65°C for 15 minutes. The reaction was cooled and 10 units of HpyCH4IV were added followed by digestion at 37°C for 20 minutes. ≥90% of the product is single cut pUC19.</p>	Pass
<p>Molecular Weight Determination (Identity) - The intact mass detected by LC-MS is ± 50 ppm of the expected mass of RecA (37,972.94 Da).</p>	Pass
<p>Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in RecA Reaction Buffer containing 1 µg of Lambda DNA and a minimum of 10 µg of RecA incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</p>	Pass
<p>Protein Concentration (A280, Range) - The concentration of RecA is from 1.9 to 2.1 mg/ml as determined by UV absorption at 280 nm.</p>	Pass



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Protein Purity Assay (SDS-PAGE) - RecA is $\geq 95\%$ 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 10 μ g of RecA 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



Authorized by
Derek Robinson
27 Apr 2018



Inspected by
Bo Wu
03 Dec 2018

