NEW ENGLAND BioLabs® Inc.

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New England Biolabs
Product Specification

Product Name:	*RecA*
Catalog #:	*M0249S/L*
Concentration:	*2 mg/ml*
Shelf Life:	*24 months*
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)

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.

Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in RecA Reaction Buffer containing 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA and a minimum of 10 µg of RecA incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

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.

Molecular Weight Determination (Identity) - The intact mass detected by LC-MS is ± 50 ppm of the expected mass of RecA (37,972.94 Da).

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.

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.

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Protein Purity Assay (SDS-PAGE) - RecA is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.
RNase Activity (Extended Digestion) - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 10 µg 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.

Date 27 Apr 2018

Derek Robinson
Director of Quality Control

ISO 9001 Registered Quality Management
ISO 14001 Registered Environmental Management
ISO 13485 Registered Medical Devices