NEW ENGLAND BioLabs Inc.
be INSPIRED drive DISCOVERY stay GENUINE
240 County Road
Ipswich, MA 01938-2723
Tel 978-927-5054
Fax 978-921-1350
www.neb.com
info@neb.com

New England Biolabs
Certificate of Analysis

Product Name:	*RecA*
Catalog Number:	*M0249S*
Concentration:	*2 mg/ml*
Packaging Lot Number:	*10128951*
Expiration Date:	*11/2023*
Storage Temperature:	*-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*

RecA Component List			
NEB Part Number	**Component Description**	**Lot Number**	**Individual QC Result**
M0249SVIAL	RecA	10128950	**Pass**
B0355SVIAL	Rec A Reaction Buffer	10122100	**Pass**

Assay Name/Specification	**Lot # 10128951**
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.	**Pass**
Protein Purity Assay (SDS-PAGE) RecA is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**
Molecular Weight Determination (Identity) The intact mass detected by LC-MS is ± 50 ppm of the expected mass of RecA (37,972.94 Da).	**Pass**
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.	**Pass**
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	**Pass**

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Assay Name/Specification	Lot # 10128951
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.	**Pass**
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 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	**Pass**
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.	**Pass**

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

Bo Wu
Production Scientist
12 Nov 2021

Michael Tonello
Packaging Quality Control Inspector
12 Nov 2021