NEW ENGLAND BioLabs® Inc.

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New England Biolabs
Product Specification

Product Name:	*RecAf*
Catalog #:	*M0355S/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 7.5 @ 25°C)*
Specification Version:	*PS-M0355S/L v1.0*
Effective Date:	*07 May 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 RecAf 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 RecAf 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 RecAf , 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. ≥95% 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 RecAf (39,038.05 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 RecAf 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 RecAf 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) - RecAf 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 RecAf is incubated at 37°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

Date 07 May 2018

Derek Robinson
Director of Quality Control