

New England Biolabs Certificate of Analysis

Product Name: RecAf
Catalog Number: M0355S
Concentration: 2 mg/ml
Packaging Lot Number: 10124320
Expiration Date: 10/2023
Storage Temperature: -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

RecAf Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0355SVIAL	RecAf	10123391	Pass
B0355SVIAL	Rec A Reaction Buffer	10122100	Pass

Assay Name/Specification	Lot # 10124320
Protein Purity Assay (SDS-PAGE) RecAf is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
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.	Pass
Molecular Weight Determination (Identity) The intact mass detected by LC-MS is ± 50 ppm of the expected mass of RecAf (39,038.05 Da).	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 RecAf incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass
Protein Concentration (A280, Range) The concentration of RecAf is from 1.9 to 2.1 mg/ml as determined by UV absorption	Pass

Assay Name/Specification	Lot # 10124320
<p>at 280 nm.</p> <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 RecAf 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] 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.</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 -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.</p>	Pass

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

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05 Nov 2021



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