

New England Biolabs Certificate of Analysis

Product Name: RecAf
Catalog Number: M0355S
Concentration: 2 mg/ml
Lot Number: 10052481
Expiration Date: 04/2021
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	10042805	Pass
B0355SVIAL	Rec A Reaction Buffer	10009781	Pass

Assay Name/Specification	Lot # 10052481
<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
<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>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.</p>	Pass

Assay Name/Specification	Lot # 10052481
Protein Purity Assay (SDS-PAGE) RecAf is $\geq 95\%$ pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
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.	Pass
Non-Specific DNase Activity (16 Hour) A 50 μ l reaction in RecA Reaction Buffer containing 1 μ g of Lambda DNA and a minimum of 10 μ g 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
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
Endonuclease Activity (Nicking) A 50 μ l reaction in RecA Reaction Buffer containing 1 μ g of supercoiled PhiX174 DNA and a minimum of 10 μ g of RecAf 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.



Bo Wu
Production Scientist
23 May 2018



Michael Tonello
Packaging Quality Control Inspector
08 Aug 2019