

## New England Biolabs Certificate of Analysis

**Product Name:** RecAf  
**Catalog Number:** M0355L  
**Concentration:** 2 mg/ml  
**Packaging Lot Number:** 10060281  
**Expiration Date:** 11/2021  
**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
M0355LVIAL	RecAf	10060280	Pass
B0355SVIAL	Rec A Reaction Buffer	10041040	Pass

Assay Name/Specification	Lot # 10060281
<b>Non-Specific DNase Activity (16 Hour)</b> 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
<b>Protein Concentration (A280, Range)</b> The concentration of RecAf is from 1.9 to 2.1 mg/ml as determined by UV absorption at 280 nm.	Pass
<b>Protein Purity Assay (SDS-PAGE)</b> RecAf is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
<b>RNase Activity (Extended Digestion)</b> 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
<b>Endonuclease Activity (Nicking)</b> A 50 µl reaction in RecA Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA	Pass

Assay Name/Specification	Lot # 10060281
<p>and a minimum of 10 µg of RecAf incubated for 4 hours at 37°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	
<p><b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in RecA Reaction Buffer containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] E. coli DNA and a minimum of 10 µg of RecAf incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p>	<b>Pass</b>
<p><b>Functional Testing (Triple Helix Formation)</b> 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>	<b>Pass</b>
<p><b>Molecular Weight Determination (Identity)</b> The intact mass detected by LC-MS is ± 50 ppm of the expected mass of RecAf (39,038.05 Da).</p>	<b>Pass</b>

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



Bo Wu  
Production Scientist  
05 Dec 2019



Michael Tonello  
Packaging Quality Control Inspector  
15 Jan 2020