

## New England Biolabs Certificate of Analysis

**Product Name:** AleI-v2  
**Catalog Number:** R0685L  
**Concentration:** 10,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in rCutSmart Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.  
**Packaging Lot Number:** 10214563  
**Expiration Date:** 10/2024  
**Storage Temperature:** -20°C  
**Storage Conditions:** 10 mM Tris-HCl, 200 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 200 µg/ml rAlbumin, 50 % Glycerol, (pH 7.4 @ 25°C)  
**Specification Version:** PS-R0685S/L v2.0

AleI-v2 Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R0685LVIAL	AleI-v2	10209086	Pass
B6004SVIAL	rCutSmart™ Buffer	10202500	Pass

Assay Name/Specification	Lot # 10214563
<p><b>Endonuclease Activity (Nicking)</b>            A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled pBR322 DNA and a minimum of 30 units of AleI-v2 incubated for 4 hours at 37°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	Pass
<p><b>Exonuclease Activity (Radioactivity Release)</b>            A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] E. coli DNA and a minimum of 100 units of AleI-v2 incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p>	Pass
<p><b>Ligation and Recutting (Terminal Integrity)</b>            After a 10-fold over-digestion of Lambda DNA with AleI-v2, &gt;95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with AleI-v2.</p>	Pass
<p><b>Non-Specific DNase Activity (16 Hour)</b>            A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 50 units of AleI-v2 incubated for 16 hours at 37°C results in a DNA pattern free of</p>	Pass


Assay Name/Specification	Lot # 10214563
detectable nuclease degradation as determined by agarose gel electrophoresis.	
<p><b>Protein Purity Assay (SDS-PAGE)</b> A1e1-v2 is <math>\geq 95\%</math> pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>qPCR DNA Contamination (E. coli Genomic)</b> A minimum of 10 units of A1e1-v2 is screened for the presence of E. coli genomic DNA using SYBR<sup>®</sup> Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is <math>\leq 1</math> E. coli genome.</p>	<b>Pass</b>

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](http://www.neb.com/trademarks) for additional information.



YunJie Sun  
Production Scientist  
04 Oct 2023



Michael Tonello  
Packaging Quality Control Inspector  
08 Jan 2024