

## New England Biolabs Certificate of Analysis

**Product Name:** *AleI-v2*  
**Catalog Number:** *R0685S*  
**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:** *10242863*  
**Expiration Date:** *05/2025*  
**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*

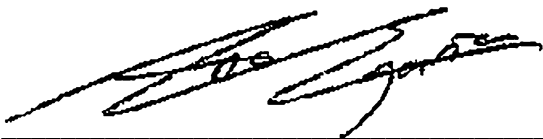
AleI-v2 Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R0685SVIAL	AleI-v2	10241137	Pass
B6004SVIAL	rCutSmart™ Buffer	10238052	Pass

Assay Name/Specification	Lot # 10242863
<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 <10% conversion to the nicked form as determined by agarose gel electrophoresis.	<b>Pass</b>
<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 <0.1% of the total radioactivity.	<b>Pass</b>
<b>Ligation and Recutting (Terminal Integrity)</b> After a 10-fold over-digestion of Lambda DNA with AleI-v2, >95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, >95% can be recut with AleI-v2.	<b>Pass</b>
<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	<b>Pass</b>

Assay Name/Specification	Lot # 10242863
detectable nuclease degradation as determined by agarose gel electrophoresis.	
<b>Protein Purity Assay (SDS-PAGE)</b> AleI-v2 is $\geq 95\%$ pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<b>Pass</b>
<b>qPCR DNA Contamination (E. coli Genomic)</b> A minimum of 10 units of AleI-v2 is screened for the presence of E. coli genomic DNA using SYBR® 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 $\leq 1$ E. coli genome.	<b>Pass</b>

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

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Ana Egana  
Production Scientist  
05 Jun 2024



Michael Tonello  
Packaging Quality Control Inspector  
05 Jun 2024