

## New England Biolabs Certificate of Analysis

**Product Name:** AlwNI  
**Catalog Number:** R0514L  
**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:** 10203101  
**Expiration Date:** 08/2025  
**Storage Temperature:** -20°C  
**Storage Conditions:** 10 mM Tris-HCl, 50 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml rAlbumin (pH 7.4 @ 25°C)  
**Specification Version:** PS-R0514S/L v2.0

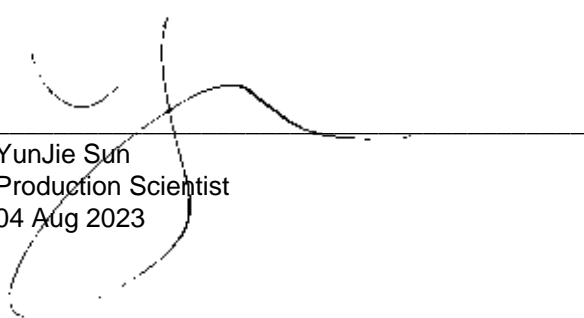
AlwNI Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R0514LVIAL	AlwNI	10201945	Pass
B6004SVIAL	rCutSmart™ Buffer	10198641	Pass

Assay Name/Specification	Lot # 10203101
<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 AlwNI incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
<b>Functional Testing (15 minute Digest)</b> A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and 1 µl of AlwNI incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.	Pass
<b>Ligation and Recutting (Terminal Integrity)</b> After a 20-fold over-digestion of Lambda DNA with AlwNI, >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 AlwNI.	Pass
<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 10 units of AlwNI incubated for 16 hours at 37°C results in a DNA pattern free of	Pass


Assay Name/Specification	Lot # 10203101
detectable nuclease degradation as determined by agarose gel electrophoresis.	
<p><b>Protein Purity Assay (SDS-PAGE)</b> AlwNI is &gt;95% 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 AlwNI 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 <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.

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04 Aug 2023



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