

New England Biolabs Certificate of Analysis

Product Name: AlwNI
Catalog Number: R0514S
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: 10233278
Expiration Date: 02/2026
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

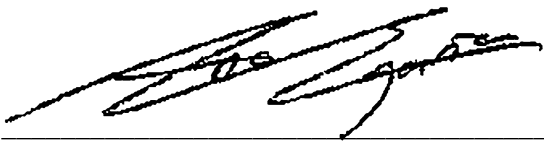
AlwNI Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R0514SVIAL	AlwNI	10228164	Pass
B6004SVIAL	rCutSmart™ Buffer	10225675	Pass

Assay Name/Specification	Lot # 10233278
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [³ 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
Functional Testing (15 minute Digest) 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
Ligation and Recutting (Terminal Integrity) 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
Non-Specific DNase Activity (16 Hour) 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 # 10233278
detectable nuclease degradation as determined by agarose gel electrophoresis.	
Protein Purity Assay (SDS-PAGE) AlwNI is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.	Pass
qPCR DNA Contamination (E. coli Genomic) 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 ≤ 1 E. coli genome.	Pass

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 for additional information.



Ana Egana
Production Scientist
26 Feb 2024



Josh Hersey
Packaging Quality Control Inspector
26 Feb 2024