

## New England Biolabs Certificate of Analysis

**Product Name:** *NotI*  
**Catalog Number:** *R0189S*  
**Concentration:** *10,000 U/ml*  
**Unit Definition:** *One unit is defined as the amount of enzyme required to digest 1 µg of pBC4 DNA in NEBuffer r3.1 in 1 hour at 37°C in a total reaction volume of 50 µl.*  
**Packaging Lot Number:** *10272275*  
**Expiration Date:** *10/2026*  
**Storage Temperature:** *-20°C*  
**Storage Conditions:** *10 mM Tris-HCl, 250 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 0.15% Triton X-100, 200 µg/ml rAlbumin (pH 7.4 @ 25°C)*  
**Specification Version:** *PS-R0189S/L/E v2.0*

NotI Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R0189SVIAL	NotI	10261387	Pass
B7024AVIAL	Gel Loading Dye, Purple (6X)	10256010	Pass
B6003SVIAL	NEBuffer™ r3.1	10250209	Pass

Assay Name/Specification	Lot # 10272275
<b>Endonuclease Activity (Nicking)</b> A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 100 units of NotI incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
<b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in NEBuffer™ r3.1 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 NotI 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 NEBuffer™ r3.1 containing 1 µg of pBC4 DNA and 1 µl of NotI 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 10-fold over-digestion of pBC4 DNA with NotI, >95% of the DNA fragments can	Pass

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<p>be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with NotI.</p>	
<p><b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of pBC4 DNA and a minimum of 100 units of NotI incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> NotI is ≥ 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 NotI 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.</p>	<b>Pass</b>

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

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