

New England Biolabs Product Specification

Product Name:	<i>NcoI</i>
Catalog #:	R0193S/L/E
Concentration:	10,000 units/ml
Unit Definition:	One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in NEBuffer r3.1 in 1 hour at 37°C in a total reaction volume of 50 µl.
Shelf Life:	24 months
Storage Temp:	-20°C
Storage Conditions:	10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml rAlbumin (pH 7.4 @ 25°C)
Specification Version:	PS-R0193S/L/E v2.0
Effective Date:	07 Oct 2022

Assay Name/Specification (minimum release criteria)

Blue-White Screening (Terminal Integrity) - A sample of LITMUS28i vector linearized with a 10-fold excess of NcoI, religated and transformed into an *E. coli* strain expressing the LacZ beta fragment gene results in <1% white colonies.

Ligation and Recutting (Terminal Integrity) - After a 5-fold over-digestion of Lambda DNA with NcoI, >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 NcoI.

Protein Purity Assay (SDS-PAGE) - NcoI is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.

Endonuclease Activity (Nicking) - A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 10 units of NcoI incubated for 4 hours at 37°C results in <50% conversion to the nicked form as determined by agarose gel electrophoresis.

Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of a mixture of single and double-stranded [³H] *E. coli* DNA and a minimum of 50 units of NcoI incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

Functional Testing (15 minute Digest) - A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of Lambda DNA and 1 µl of NcoI incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.

Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of Lambda DNA and a minimum of 10 units of NcoI incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.



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qPCR DNA Contamination (<i>E. coli</i> Genomic) - A minimum of 10 units of NcoI is screened for the presence of <i>E. coli</i> genomic DNA using SYBR® Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is ≤ 1 <i>E. coli</i> genome.

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Nancy Considine

Date 07 Oct 2022

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Quality Approver

