

New England Biolabs Certificate of Analysis

Product Name: NcoI
Catalog #: R0193T/M
Concentration: 50,000 units/ml
Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in 1 hour at 37°C in a total reaction volume of 50 µl.
Lot #: 0311311
Assay Date: 11/2013
Expiration Date: 11/2015
Storage Temp: -20 °C
Storage Conditions: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml BSA
Specification Version: PS-R0193T/M v1.0
Effective Date: 18 Oct 2013

| Assay Name/Specification (minimum release criteria) | Lot #0311311 |
|---|--------------|
| Blue-White Screening (Terminal Integrity) - A sample of LITMUS28i vector linearized with a 10-fold excess of NcoI, religated and transformed into an <i>E. coli</i> strain expressing the LacZ beta fragment gene results in <1% white colonies. | Pass |
| Endonuclease Activity (Nicking) - A 50 µl reaction in NEBuffer 3.1 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. | Pass |
| Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in NEBuffer 3.1 containing 1 µg of a mixture of single and double-stranded [³ H] <i>E. coli</i> DNA and a minimum of 50 units of NcoI incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. | Pass |
| 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. | Pass |
| Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in NEBuffer 3.1 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. | Pass |
| Protein Purity Assay (SDS-PAGE) - NcoI is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection. | Pass |



240 County Road
Ipswich, MA 01938-2723

Tel 978-927-5054
Fax 978-921-1350

www.neb.com
info@neb.com

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* The BSA in this product has been granted an EDQM "Certificate of Suitability" from the European Directorate for the Quality of Medicines (# R1-CEP-2003-204-Rev00) and has been granted a USDA Certificate for Export of Bovine Blood Plasma/Serum for Manufacture into Pharmaceutical Products.

M. W. Southworth

Authorized by
Maurice Southworth
18 Oct 2013

C. Madinger

Inspected by
Casey Madinger
20 Dec 2013

