

240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

New England Biolabs Certificate of Analysis

| Product Name: | SacI |
|------------------------|---|
| Catalog #: | R0156M |
| Concentration: | 100,000 units/ml |
| Unit Definition: | One unit is defined as the amount of enzyme required to digest 1 μ g of Lambda DNA (HindIII digest) in 1 hour at 37°C in a total reaction volume of 50 μ l. |
| Lot #: | 0501602 |
| Assay Date: | 02/2016 |
| Expiration Date: | 2/2018 |
| Storage Temp: | -20°C |
| Storage Conditions: | 100 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 μg/ml BSA |
| Specification Version: | PS-R0156M v1.0 |
| Effective Date: | 26 Jul 2013 |

| Assay Name/Specification (minimum release criteria) | Lot #0501602 |
|---|--------------|
| Blue-White Screening (Terminal Integrity) - A sample of LITMUS28i vector linearized with a 10-fold excess of SacI, 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 μ l reaction in NEBuffer 1.1 containing 1 μ g of supercoiled PhiX174 DNA and a minimum of 20 units of SacI incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis. | Pass |
| Exonuclease Activity (Radioactivity Release) - A 50 μ l reaction in NEBuffer 1.1 containing 1 μ g of a mixture of single and double-stranded [³ H] <i>E. coli</i> DNA and a minimum of 100 units of SacI incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. | Pass |
| Ligation and Recutting (Terminal Integrity) - After a 20-fold over-digestion of pXba DNA with SacI, >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 SacI. | Pass |
| Non-Specific DNase Activity (16 Hour) - A 50 μ l reaction in NEBuffer 1.1 containing 1 μ g of Lambda- HindIII DNA and a minimum of 60 units of SacI 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) - SacI is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection. | Pass |



R0156M Lot: 0501602 Page 1 of 2



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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.

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Authorized by Derek Robinson 26 Jul 2013



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Inspected by Anthony Francis 26 Feb 2016