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## New England Biolabs Certificate of Analysis

Product Name: I-SceI
Catalog #: R0694S/L
Concentration: 5,000 units/ml

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 µg of pGPS2 NotI-Inearized Control Plasmid in 1 hour at

 $37^{\circ}C$  in a total reaction volume of  $50 \mu l$ .

Lot #:0121411Assay Date:11/2014Expiration Date:11/2016Storage Temp:-80 °C

Storage Conditions: 300 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 500 µg/ml BSA

Specification Version: PS-R0694S/L v1.0
Effective Date: 28 Feb 2014

Assay Name/Specification (minimum release criteria)	Lot #0121411
Endonuclease Activity (Nicking) - A 50 μl reaction in CutSmart <sup>TM</sup> Buffer containing 1 μg of supercoiled PhiX174 DNA and a minimum of 15 units of I-SceI incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
<b>Exonuclease Activity (Radioactivity Release)</b> - A 50 μl reaction in CutSmart™ Buffer containing 1 μg of a mixture of single and double-stranded [ <sup>3</sup> H] <i>E. coli</i> DNA and a minimum of 50 units of I-SceI incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
<b>Ligation and Recutting (Terminal Integrity)</b> - After a 10-fold over-digestion of pGPS2-NotI DNA with I-SceI, >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 I-SceI.	Pass
Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in CutSmart <sup>TM</sup> Buffer containing 1 µg of pGPS2-NotI DNA and a minimum of 50 units of I-SceI incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass

Authorized by Derek Robinson 28 Feb 2014

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Inspected by Jianying Luo 14 Nov 2014

<sup>\*</sup> 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.