NEW ENGLAND BioLabs Inc.
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New England Biolabs
Certificate of Analysis

Product Name:	*I-SceI*
Catalog Number:	*R0694L*
Concentration:	*5,000 U/ml*
Unit Definition:	*One unit is defined as the amount of enzyme required to cleave 1 µg of pGPS2 NotI-linearized Control Plasmid in rCutSmart Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.*
Packaging Lot Number:	*10179261*
Expiration Date:	*02/2025*
Storage Temperature:	*-80°C*
Storage Conditions:	*10 mM Tris-HCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 500 µg/ml rAlbumin (pH 7.4 @ 25°C)*
Specification Version:	*PS-R0694S/L v2.0*

I-SceI Component List

NEB Part Number	Component Description	Lot Number	Individual QC Result
R0694LVIAL	I-SceI	10179260	**Pass**
N0420SVIAL	pGPS2 NotI-linearized Control Plasmid	10179265	**Pass**
B6004SVIAL	rCutSmart™ Buffer	10173663	**Pass**

Assay Name/Specification	Lot # 10179261
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [^{3}H] E. coli 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**
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of pGPS2-NotI DNA and a minimum of 50 units of I-SceI incubated for 16 hours at 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	**Pass**
Endonuclease Activity (Nicking) A 50 µl reaction in rCutSmart™ 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**
Protein Purity Assay (SDS-PAGE)	**Pass**

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Assay Name/Specification	Lot # 10179261
I-SceI is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	
Ligation and Recutting (Terminal Integrity) 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 16ºC. Of these ligated fragments, >95% can be recut with I-SceI.	**Pass**
qPCR DNA Contamination (E. coli Genomic) A minimum of 5 units of I-SceI 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.	**Pass**

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

YunJie Sun
Production Scientist
03 Feb 2023

Michael Tonello
Packaging Quality Control Inspector
08 Feb 2023