

## New England Biolabs Certificate of Analysis

**Product Name:** I-SceI  
**Catalog Number:** R0694S  
**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:** 10156854  
**Expiration Date:** 06/2024  
**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
R0694SVIAL	I-SceI	10156746	Pass
N0420SVIAL	pGPS2 NotI-linearized Control Plasmid	10156707	Pass
B6004SVIAL	rCutSmart™ Buffer	10156426	Pass

Assay Name/Specification	Lot # 10156854
<b>Endonuclease Activity (Nicking)</b> 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
<b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> 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
<b>qPCR DNA Contamination (E. coli Genomic)</b> 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
<b>Non-Specific DNase Activity (16 Hour)</b>	Pass

Assay Name/Specification	Lot # 10156854
<p>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 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</p>	
<p><b>Protein Purity Assay (SDS-PAGE)</b> I-SceI is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>Ligation and Recutting (Terminal Integrity)</b> After a 10-fold over-digestion of pGPS2-NotI DNA with I-SceI, &gt;95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with I-SceI.</p>	<b>Pass</b>

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

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05 Aug 2022




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05 Aug 2022