

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: *10179263*
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	10179259	Pass
N0420SVIAL	pGPS2 NotI-linearized Control Plasmid	10156707	Pass
B6004SVIAL	rCutSmart™ Buffer	10175291	Pass

Assay Name/Specification	Lot # 10179263
<p>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.</p>	Pass
<p>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 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</p>	Pass
<p>Exonuclease Activity (Radioactivity Release) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [³H] 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.</p>	Pass

Assay Name/Specification	Lot # 10179263
<p>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.</p>	Pass
<p>Protein Purity Assay (SDS-PAGE) I-SceI is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	Pass
<p>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 16°C. Of these ligated fragments, >95% can be recut with I-SceI.</p>	Pass

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

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01 Aug 2022



Michael Tonello
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16 Feb 2023