# I-SceI

1-800-632-7799
info@neb.com
www.neb.com

R0694S 011120914091

# R0694S

RR NEBU
BSA 37° Yes

| | | |
|---|---|---|
| **500 units** | **5,000 U/ml** | **Lot: 0111209** |
| **RECOMBINANT** | **Store at –80°C** | **Exp: 9/14** |

**Description:** I-SceI is an intron-encoded endonuclease. The intron encoding I-SceI is present in mitochondria of *Saccharomyces cerevisiae.*

**Source:** An *E. coli* strain that carries the cloned I-SceI mitochondrial gene from *Saccharomyces cerevisiae* (B. Dujon)

**More Units**

**Note:** Homing endonucleases do not have stringently-defined recognition sequences in the way that restriction enzymes do. That is, single base changes do not abolish cleavage but reduce its efficiency to variable extents. The precise boundary of required bases is generally not known. The recognition sequence listed is one site that is known to be recognized and cleaved.

**Specificity:** The homing or recognition site for this endonuclease is shown below:

```
5´...TAGGGATAACAGGGTAAT...3´
3´...ATCCCTATTGTCCCATTA...5´
```

Double-stranded cleavage at the site indicated by arrows yields a four base, 3´ extension. The sequence degeneracy tolerated by this enzyme has not yet been determined.

Supplied in: 300 mM NaCl, 10 mM Tris-HCl (pH 7.4 ), 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol.

***Storage Note:** Using this product to study transgenesis requires the enzyme to be stored at –80°C. For simple DNA digestions, this product can be stored at –20°C. See the following reference for more information: Rembold, M. et al. (2006) *Nature Protocols* 1, 1133–1139.

**Reagents Supplied with Enzyme:**
10X NEBuffer I-SceI, 100X BSA, 5 µg pGPS2 NotI-inearized Control Plasmid.

**Reaction Conditions:** 1X NEBuffer I-SceI, supplemented with 100 µg/ml BSA. Incubate at 37°C.

**1X NEBuffer I-SceI:**
10 mM Tris-HCl
10 mM $MgCl_2$
1 mM DTT
pH 8.8 @ 25°C

**Unit Definition:** One unit is defined as the amount of enzyme required to cleave 1 µg of pGPS2 NotI-linearized Control Plasmid in 1 hour at 37°C in a total reaction volume of 50 µl.

**Diluent Compatibility:** Diluent Buffer B
300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol. (pH 7.4 @ 25°C)

## Quality Control Assays

**Ligation and Re-cutting:** After a 10-fold overdigestion with I-SceI, > 95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5´ termini concentration of 1–2 µM) at 16°C. Of these ligated fragments, > 95% can be recut with I-SceI.

**16-Hour Incubation:** A 50 µl reaction containing 1 µg of DNA and 50 units of I-SceI incubated for 16 hours at 37°C resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**Exonuclease Activity:** Incubation of a 50 µl reaction containing 50 units of I-SceI with 1 µg of a mixture of single and double-stranded [$^3$H] *E. coli* DNA ($20^5$ cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

*(see other side)*

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

**Endonuclease Activity:** Incubation of a 50 µl reaction containing 25 units of I-SceI with 1 µg of ϕX174 RF I DNA for 4 hours at 37°C resulted in < 20% conversion to RFII as determined by agarose gel electrophoresis

**Plasmid DNA:** pGPS2 NotI-linearized Control Plasmid is supplied at 0.5 mg/ml in 10 mM Tris-HCl (pH 8.0) and1 mM EDTA. Cleavage of this 2,499 bp plasmid with I-SceI gives fragments of 1,518 and 981 base pairs.

## Enzyme Properties

**Activity in NEBuffers:**

| | |
|---|---|
| NEBuffer 1 | 10% |
| NEBuffer 2 | 50% |
| NEBuffer 3 | 50% |
| NEBuffer 4 | 50% |

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

**Activity in Unique Homing Endonuclease Buffers:**

| | |
|---|---|
| NEBuffer I-SceI | **100%** |
| NEBuffer PI-PspI | 50% |
| NEBuffer PI-SceI | 25% |

**Heat Inactivation:** 50 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

**Note:** For additional information about homing endonucleases, visit www.neb.com.

**References:**


1. Monteilhet, C. et al. (1990) *Nucleic Acids Res.* 18, 1407–1413.
2. Colleaux, L. et al. (1988) *Proc. Natl. Acad. Sci. USA* 85, 6022–6026.




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