I-CeuI

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

R0699S 008120914091

R0699S

RR NEB4 BSA 37° Yes

500 units **5,000 U/ml** **Lot: 0081209**

RECOMBINANT **Store at –20°C** **Exp: 9/14**

Description: I-CeuI is an intron-encoded endonuclease. The intron encoding I-CeuI is present in the chloroplast large rRNA gene of *Chlamydomonas eugametos* (1). This gene has been cloned and overexpressed in *E. coli* (2).

Source: An *E. coli* strain that carries the cloned I-CeuI gene from *Chlamydomonas eugametos* (Claude Lemieux) (2)

New Storage Conditions

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

```
5´...TAACTATAACGGTCCTAAGGTAGCGA...3´
3´...ATTGATATTGCCAGGATTCCATCGCT...5´
```

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.

Double-stranded cleavage at the site indicated by arrows yields a four-base 3´ extension (2). Some sequence degeneracy is tolerated within this sequence. As a result, their observed sequence specificity is typically in the range of 10–12 base pairs.

Supplied in: 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)

Reagents Supplied with Enzyme:
10X NEBuffer 4, 100X BSA,
5 µg pBHS ScaI-linearized Control Plasmid.

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 µg/ml BSA. Incubate at 37°C.

1X NEBuffer 4:
50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
1 mM dithiothreitol
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 µg of pBHS ScaI-linearized Control Plasmid in 3 hours 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: Cleavage by I-CeuI leaves DNA fragments with four nucleotide 3´ extensions. Fragments with complimentary ends can be joined by T4 DNA Ligase. After 20-fold overdigestion with I-CeuI, > 95% of the fragments could be ligated and recut with this enzyme.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 50 units of enzyme incubated for 16 hours at 37°C resulted in the same pattern of bands as a reaction produced in 3 hours with one unit of enzyme.

Exonuclease Activity: Incubation of 50 units for 4 hours at 37°C in 50 µl assay buffer with 1 µg sonicated ^{3}H DNA (10^{5} cpm/µg) released < 0.1% radioactivity.

Endonuclease Activity: Incubation of a 50 µl reaction containing 25 units of I-CeuI 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.

(see other side)

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

Plasmid DNA: pBHS ScaI-linearized Control Plasmid is supplied 0.5 mg/ml in 10 mM Tris-HCl (pH 8.0), 1 mM EDTA. Cleavage of this 3.5 kb plasmid gives fragments of 2100 and 1400 base pairs.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	10%
NEBuffer 2	10%
NEBuffer 3	0%
NEBuffer 4	**100%**

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	25%
NEBuffer PI-PspI	10%
NEBuffer PI-SceI	50%

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

Notes: I-CeuI can remain bound to DNA after cutting and alter migration rate of DNA during electrophoresis. To disrupt binding, add SDS to a final concentration of 0.5% or purify DNA before electrophoresis.

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

References:

1. Gauthier, A. et al. (1991) *Curr. Genet.* 19, 43–47.
2. Marshall, P. and Lemieux, C. (1991) *Gene* 104, 241–245.
3. Marshall, P. and Lemieux, C. (1992) *Nucl. Acids Res.* 20, 6401–6407.
4. Liu, S. et al. (1993) *Proc. Natl. Acad. Sci. USA* 90, 6874–6878.
5. Marshall, P. et al. (1994) *Eur. J. Biochem.* 220, 855–859.

U.S. Patent No. 5,420,032

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References:

1. Gauthier, A. et al. (1991) *Curr. Genet.* 19, 43–47.
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