PI-SceI

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

R0696S 012120914091

R0696S

RR NEBU
BSA 37° Yes

250 units **5,000 U/ml** **Lot: 0121209**
RECOMBINANT **Store at –20°C** **Exp: 9/14**

Description: The intein encoding PI-SceI is present in the VMA ATPase gene *Saccharomyces cerevisiae* (1,5). The gene has been modified for independent expression in *E. coli* using a T7 RNA polymerase expression system (2).

Source: An *E. coli* strain that carries the cloned VMA1 ATPase gene from *Saccharomyces cerevisiae* (2)

More Units, New Storage Conditions

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

5´ATCTATGTCGGGTGC▼GGAGAAAGAGGTAATGAAATGG 3´
3´TAGATACAGCC▲CACGCCTCTTTCTCCATTACTTTACC 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. The sequence degeneracy tolerated by this enzyme has not yet been determined. However, digestion patterns from bacterial and yeast chromosomal DNAs indicate that the observed sequence specificity is greater than 11 bases (3).

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 PI-SceI, 100X BSA,
5 µg pBSvdeX XmnI-linearized Control Plasmid.

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

1X NEBuffer PI-SceI :
100 mM KCl
10 mM Tris-HCl
10 mM $MgCl_2$
1 mM DTT
pH 8.6 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 µg of pBSvdeX XmnI-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 and Recutting: Cleavage by PI-SceI leaves DNA fragments with four nucleotide 3´ extensions. Fragments with complimentary ends can be joined by T4 DNA Ligase. After 10-fold overdigestion with PI-SceI , > 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 resulted in the same pattern of DNA bands as a reaction incubated for 3 hours with 1 unit of enzyme.

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

Plasmid DNA: pBSvdeX XmnI-linearized Control Plasmid is supplied 0.5 mg/ml in 10 mM Tris-HCl (pH 8.0), 1 mM EDTA. Cleavage of this 3.7 kb plasmid with PI-Sce I gives fragments of 2550 and 1150 base pairs.

(see other side)

CERTIFICATE OF ANALYSIS

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Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	0%
NEBuffer 2	0%
NEBuffer 3	0%
NEBuffer 4	0%

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	0%
NEBuffer PI-PspI	10%
NEBuffer PI-SceI	**100%**

Heat Inactivation: 10 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Notes: PI-SceI 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. Hirata, R. et al. (1990) *J. Biol. Chem.* 265, 6726–6733.
2. Gimble, F.S. and Thorner, J. (1992) *Nature* 357, 301–306.
3. Gimble, F.S. et al. (1993) *J. Biol. Chem.* 268, 21844–21853.
4. Bremer, M.C.D. et al. (1992) *Nucleic Acids Res.* 20, 5484.
5. Kane, P.M. et al. (1990) *Science* 250, 651–657.

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