PI-PspI

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

R0695S 003120914091

R0695S

500 units **5,000 U/ml** **Lot: 0031209**

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

Description: PI-PspI is obtained from a strain of *E. coli* which expresses the DNA polymerase from the extreme thermophile, *Pyrococcus* species GB-D (1). The endonuclease is a product of *in vivo* protein splicing that gives rise to both polymerase and endonuclease from a single polypeptide precursor.

Source: An *E. coli* strain that carries the cloned PI-PspI gene from *Pyrococcus* species (H.W. Jannasch)

New Storage Conditions

Specificity: The homing or recognition site for this endonuclease is shown below (2):

```
5´...TGGCAAACAGCTATTATGGGTATTATGGGT...3´
3´...ACCGTTTGTCGATAATACCCATAATACCCA...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 8–10 bases under stringently controlled conditions.

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-PspI, 100X BSA,
5 µg pAKR7 XmnI-linearized Control Plasmid.

Reaction Conditions: 1X NEBuffer PI-PspI, supplemented with 100 µg/ml BSA. **Incubate at 65°C.**

1X NEBuffer PI-PspI:
150 mM KCl
10 mM Tris-HCl
10 mM $MgCl_2$
1 mM DTT
(pH 9.2 @ 25°C)

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 µg of pAKR7 XmnI-linearized Control Plasmid in 1 hour at 65°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-PspI leaves DNA fragments with four nucleotide 3´ extensions. Fragments with complimentary ends can be joined by T4 DNA Ligase. After 5-fold overdigestion with PI-PspI, > 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 5 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme.

Exonuclease Activity: Incubation of 50 units for 4 hours at 65°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 15 units of PI-PspI with 1 µg of ϕX174 RF I DNA for 4 hours at 65°C resulted in < 20% conversion to RFII as determined by agarose gel electrophoresis.

(see other side)

CERTIFICATE OF ANALYSIS

PI-PspI

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

R0695S 003120914091

R0695S

500 units 5,000 U/ml Lot: 0031209

RECOMBINANT Store at –20°C Exp: 9/14

Description: PI-PspI is obtained from a strain of *E. coli* which expresses the DNA polymerase from the extreme thermophile, *Pyrococcus* species GB-D (1). The endonuclease is a product of *in vivo* protein splicing that gives rise to both polymerase and endonuclease from a single polypeptide precursor.

Source: An *E. coli* strain that carries the cloned PI-PspI gene from *Pyrococcus* species (H.W. Jannasch)

New Storage Conditions

Specificity: The homing or recognition site for this endonuclease is shown below (2):

5´...TGGCAAACAGCTATTATGGGTATTATGGGT...3´
3´...ACCGTTTGTCGATAATACCCATAATACCCA...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 8–10 bases under stringently controlled conditions.

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-PspI, 100X BSA,
5 µg pAKR7 XmnI-linearized Control Plasmid.

Reaction Conditions: 1X NEBuffer PI-PspI, supplemented with 100 µg/ml BSA. Incubate at 65°C.

1X NEBuffer PI-PspI:
150 mM KCl
10 mM Tris-HCl
10 mM $MgCl_2$
1 mM DTT
(pH 9.2 @ 25°C)

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 µg of pAKR7 XmnI-linearized Control Plasmid in 1 hour at 65°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-PspI leaves DNA fragments with four nucleotide 3´ extensions. Fragments with complimentary ends can be joined by T4 DNA Ligase. After 5-fold overdigestion with PI-PspI, > 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 5 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme.

Exonuclease Activity: Incubation of 50 units for 4 hours at 65°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 15 units of PI-PspI with 1 µg of ϕX174 RF I DNA for 4 hours at 65°C resulted in < 20% conversion to RFII as determined by agarose gel electrophoresis.

(see other side)

CERTIFICATE OF ANALYSIS

Plasmid DNA: pAKR7 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 gives fragments of 2146 and 1532 base pairs.

Enzyme Properties

Activity in NEBuffers:

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

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	10%
NEBuffer PI-PspI	**100%**
NEBuffer PI-SceI	25%

Heat Inactivation: No

Notes: Digests of DNA embedded in agarose should be performed with 1 unit of enzyme per µg of DNA for 3 hours at 50°C.

PI-PspI 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.

Incubation at 37° results in 5% activity.

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

References:

1. Xu, Ming-Qun, et al. (1993) *Cell* 75, 1371.
2. Davis, T.B., unpublished observations.

Plasmid DNA: pAKR7 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 gives fragments of 2146 and 1532 base pairs.

Enzyme Properties

Activity in NEBuffers:

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

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	10%
NEBuffer PI-PspI	**100%**
NEBuffer PI-SceI	25%

Heat Inactivation: No

Notes: Digests of DNA embedded in agarose should be performed with 1 unit of enzyme per µg of DNA for 3 hours at 50°C.

PI-PspI 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.

Incubation at 37° results in 5% activity.

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

References:

1. Xu, Ming-Qun, et al. (1993) *Cell* 75, 1371.
2. Davis, T.B., unpublished observations.

Page 2 (R0695)