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200 units 10,000 U/ml Lot: 0031209 RECOMBINANT Store at -20°C Exp: 9/14

#### **Recognition Site:**

5′... A CATGT...3′ 3′... TGTACA...5′

**Source:** An *E. coli* strain that carries the cloned Pcil gene from *Planococcus citreus* SE-F45 (S.K. Degtyarev).

**New Storage Conditions** 



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5′.... A<sup>T</sup>C AT G T .... 3′ 3′... T G T A C<sub>A</sub> A .... 5′

**Source:** An *E. coli* strain that carries the cloned Pcil gene from *Planococcus citreus* SE-F45 (S.K. Degtyarev). Supplied in: 300 mM NaCl, 10 mM Tris-HCl (pH 7.4 @ 25°C), 0.1 mM EDTA, 1 mM DTT, 500 µg/ ml BSA and 50% glycerol.

**Reagents Supplied with Enzyme:** 10X NEBuffer 3, 100X BSA

**Reaction Conditions:** 1X NEBuffer 3, supplemented with 100 μg/ml BSA. Incubate at 37°C.

### 1X NEBuffer 3:

100 mM NaCl 50 mM Tris-HCl 10 mM MgCl<sub>2</sub> 1 mM DTT pH 7.9 @ 25°C

**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 µg of pXba DNA 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 7.5 @ 25°C).

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## 1X NEBuffer 3:

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**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 7.5 @ 25°C).

## Quality Control Assays

**Ligation:** After a 10-fold overdigestion with Pcil, > 95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5' termini concentration of 1-2  $\mu$ M) at 16°C. Of these ligated fragments, > 95% can be recut with Pcil.

**16-Hour Incubation:** A 50  $\mu$ I reaction containing 1  $\mu$ g of DNA and 50 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 100 units of enzyme with 1  $\mu$ g sonicated [<sup>3</sup>H] DNA (10<sup>5</sup> cpm/  $\mu$ g) for 4 hours at 37°C in 50  $\mu$ l reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 30 units of enzyme with 1  $\mu$ g of  $\phi$ X174 RF I DNA for 4 hours at 37°C in 50  $\mu$ I reaction buffer resulted in < 10% conversion to RF II.

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# Enzyme Properties

Activity in NEBuffers: NEBuffer 1 50%

 NEBuffer 1
 50%

 NEBuffer 2
 75%

 NEBuffer 3
 100%

 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.

Survival in a Reaction: A minimum of 0.25 unit is required to digest 1  $\mu g$  of substrate DNA in 16 hours.

Heat Inactivation: 80°C for 20 minutes.

Notes: Pcil is an isoschizomer of Bsp LU111.

Not sensitive to *dam, dcm* or mammalian CpG methylation.

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NEBuffer 1	50%
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NEBuffer 3	100%
NEBuffer 4	50%

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