Methyl-directed DNA Endonuclease

# Pcs I

## E505

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**50 u** **Lot: 3**

**1000 u/ml** **Store at -20°C**

**Recognition Sequence:**

**5'- (5mC)GNNNNN↓NN(5mC)G- 3'**
**3'- G(5mC)NN↑NNNNNG(5mC)- 5'**

**Sourse:** ***Paracoccus carotinifaciens 3K***

**The enzyme cleaves only C5-methylated DNA and does not cut unmodified DNA.**

**Warranty period for the enzyme storage at-20˚C is two years from the date of the last assay indicated on the enzyme vial.**

**Supplied in:**
10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0,1 mM EDTA; 100 µg/ml BSA; 7 mM 2-mercaptoethanol; 50% glycerol.

**Reaction Conditions**:
1×SEBuffer **PcsI**
Incubate at **37°C**.

**1×SEBuffer PcsI** (pH 8.3 @ 25ºC)
10 mM Tris-HCl 20 mM NaCl
3 mM $MgCl_2$ 1 mM DTT

**Unit Definition:**
One unit is defined as the amount of enzyme required to digest a unique site 5`-A(5mC)GNNNNNNN(5mC)GT-3` in 1 µg of DNA pMHgal/DriI in 1 hour at 37°C in a total reaction volume of 50 µl.

**DNA pMHgal/DriI** is a linearized plasmid pMHgaI, which included a genes of DNA-methyltransferases M1.HgaI (recognition sequence 5'-GCGTC-3') and M2.HgaI (5'-GACGC-3') and contains a unique PcsI canonical site: 5'-W(5mC)GNNNNNNN(5mC)GW-3'/3'-WG(5 m C)NNNNNNNG(5mC)W-5'.

The enzyme activity depends on number and position of methylated nucleotides in the recognition sequence.
**Optimal recognition site** (100% activity ):
**5'-W(5mC)GNNNNNNN(5mC)GW-3'/ 3'-WG(5mC)NNNNNNNG(5mC)W-5`**

**Quality Control Assays**

**16-Hour Incubation:** No detectable degradation of 1 µg of λ DNA was observed after incubation with 1 units of enzyme for 16 hours at 37ºC

**Oligonucleotide Assay:**
No detectable degradation of a single- and double-stranded oligonucleotide was observed after incubation with 1 units of enzyme for 3 hours.

**Enzyme Properties**
**Activity in SEBuffers**:
SEBuffer B 50-75%
SEBuffer G 25-50%
SEBuffer O 0%
SEBuffer W 10-25**%**
SEBuffer Y 50-75%
SEBuffer ROSE 20%

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

**Reagents Supplied with Enzyme:** 10×SEBuffer PcsI
**Heat Inactivation: Yes** (65°C for 20 minutes)

**CERTIFICATE OF ANALYSIS**