Methyl-directed DNA Endonuclease

Aox I

E569

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50 u **Lot: 2**

500 u/ml **Store at -20°C**

Recognition Sequence:

5'…↓PuG(5mC)Py …3'
3'… Py(5mC)GPu↑…5'

Sourse: ***Arthrobacter oxydans 25K***

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

Supplied in:
10 mM KH_2PO_4 (pH 7.4); 100 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 50% glycerol.

Reaction Conditions:

1×SEBuffer **Aox I** Incubate at **60°C**.

1×SEBuffer Aox I (pH 8.5 @ 25ºC)
10 mM Tris-HCl 3 mM $MgCl_2$ 1 mM DTT

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

Unit Definition: One unit is defined as the amount of enzyme required to hydrolyze in 1 µg of linearized plasmid pMHaeIII/DriI in 1 hour at 60°C in a total reaction volume of 50 µl.

DNA pMHaeIII/DriI is a linearized plasmid pMHaeIII. pMHaeIII carries a gene of DNA-methyltransferase M.HaeIII, which methylates sites 5`-GGCC-3` producing 5`- GG(5mC)C-3`/3`-C(5mC)GG-5`.

Quality Control Assays

16-Hour Incubation:
No detectable degradation of 1 µg of Lambda DNA was observed after incubation with 1 units of enzyme for 16 hours at 60°C in a total reaction volume of 50 µl.

Oligonucleotide Assay:
No detectable degradation of a single- and double-stranded oligonucleotide was observed after incubation with 0,5 units of enzyme for 3 hours.

Activity in SEBuffers:

SEBuffer B	75-100%
SEBuffer G	25-50%
SEBuffer O	10-25%
SEBuffer W	25-50%
SEBuffer Y	75-100%
SEBuffer ROSE	100%

Reagents Supplied with Enzyme: 10×SEBuffer Aox I
Heat Inactivation: **No** (80°C for 20 minutes)

CERTIFICATE OF ANALYSIS