Restriction Endonuclease

Bar I

E548

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500 u **Lot: 7**
2000 u/ml **Store at -20°C**

Recognition Sequence:

5'… ↓$(N)_7$GAAG$(N)_6$TAC$(N)_{12}$↓…3'
3'… ↑$(N)_{12}$CTTC$(N)_6$ATG$(N)_7$↑… 5'

Sourse: ***Bacillus sphaericus***

Supplied in: 20 mM KH_2PO_4 (pH 7.4); 100 mM KCl; 0.1 mM EDTA; 200 μg/ml BSA, 7 mM 2-mercaptoethanol; 50% glycerol.

Reaction Conditions:
1×SEBuffer **2K**
Incubate at **37°C**.

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

1×SEBuffer 2K (pH 7.6 @ 25ºC)
10 mM Tris-HCl 200 mM KCl
10 mM $MgCl_2$ 1 mM DTT

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 μg of T7 DNA in 1 hour at 37°C in a total reaction volume of 50 μl.

Quality Control Assays

Ligation: After 3-fold overdigestion with Bar I, ~90% of T7 DNA fragments can be ligated with T4 DNA Ligase and ~95% of these can be recut.

16-Hour Incubation:
A 50 μl reaction containing 1μg of T7 DNA and 4 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour.

Oligonucleotide Assay:
No detectable degradation of a single- and double-stranded oligonucleotide was observed after incubation with 2 units of enzyme for 3 hours.

Enzyme Properties

Activity in SEBuffers:
SEBuffer B 0%
SEBuffer G 0-10%
SEBuffer O 25-50%
SEBuffer W 50-75%
SEBuffer Y 10-25%
SEBuffer ROSE 40%
When using a buffer other than the optimal (suppied) SEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

Heat Inactivation:
Yes (65°C for 20 minutes)

Reagents Supplied with Enzyme:
10×SEBuffer 2K

CERTIFICATE OF ANALYSIS