Restriction Endonuclease



E131



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100 u 1000 u/ml Lot: 24 Store at -20°C

Recognition Sequence:

5'... ↓(N)₇GAAC(N)₆TAC(N)₁₂↓...3' 3'... ↑(N)₁₂CTTG(N)₆ATG(N)₇↑...5'

Sourse: Pseudomanas stutzeri N2

Supplied in:

10 mM Tris-HCl (pH 7.5); 50 mM KCl; 0,1 mM EDTA; 200 μg/ml BSA; 7 mM 2-mercaptoethanol; 50% glycerol.

Reaction Conditions:

1×SEBuffer Y+BSA

Incubate at 30°C.

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

1×SEBuffer Y (pH 7.9 @ 25°C)

33 mM Tris-Ac 66 mM KAc 10 mM MgAc 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 30°C in a total reaction volume of 50 μl.

in 1 hour at 30°C in a total reaction volume of 50 μ l. Concentrated enzymes are diluted to approximately 1000 units/ml with the buffer A [10 mM Tris-HCl (pH 7.6); 50 mM KCl; 0.1 mM EDTA; 1 mM DTT; 200 μ g/ml BSA; 50% glycerol] before determining their activity. To obtain 100% activity, BSA should be added to the 1× reaction mix to a final concentration of 100 μ g/ml.

Quality Control Assays

Ligation: After 3-fold overdigestion with Psr I, ~70% of T7 DNA fragments can be ligated with T4 DNA Ligase and ~90% of these can be recut. In the presence of 10% PEG ligation is better.

16-Hour Incubation:

A 50 μ I reaction containing 1 μ g of T7 DNA and 1 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour.

No using BSA for long incubation.

High enzyme concentration may result in star activity.

Oligonucleotide Assay:

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

Incubation at 37°C results in 20% activity.

Enzyme Properties

Activity in SEBuffers:

 SEBuffer B
 10-25%

 SEBuffer G
 10-25%

 SEBuffer O
 0%

 SEBuffer W
 0-10%

 SEBuffer Y
 100%

 SEBuffer ROSE
 30%

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 Y, BSA (10 mg/ml)

CERTIFICATE OF ANALYSIS