Restriction Endonuclease

Afe I

E213

SibEnzyme Ltd., Russia
Ph: +7 383 333 4991
Fax :+7 383 333 6853
info@sibenzyme.com
www.sibenzyme.com

200 u **Lot:**

1000 u/ml **Store at -20°C**

Recognition Sequence:

5'… AGC↓GCT …3'
3'… TCG↑CGA …5'

Sourse: An *E.coli* strain that carries the cloned Afe I gene from *Alcaligenes faecalis* T2774.

Supplied in:
10 mM Tris-HCl (pH 7.6); 50 mM NaCl; 0,1 mM EDTA; 200 μg/ml BSA; 1mM DTT; 50% glycerol.

Reaction Conditions:
1×SEBuffer **Y**
Incubate at 37°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

33 mM Tris-Ac (pH 7.9 @ 25ºC) 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 λ DNA (BamH I-digest) in 1 hour at 37 ºC in a total reaction volume of 50 μl.

Quality Control Assays

Ligation: After 10-fold overdigestion with Afe I, approximately 80% of DNA pBR322 fragments can be ligated with T4 DNA Ligase and recut.

16-Hour Incubation:
A 50 μl reaction containing 1μg of λ DNA and 10 units of enzyme incubated for 16 hours resulted in the same pattern of DNA band as a reaction incubated for 1 hour.

Oligonucleotide Assay:

No detectable degradation of a single- and double-stranded oligonucleotide was observed after incubation with 10 units of enzyme for 3 hours.

Enzyme Properties

Activity in SEBuffers:

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

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.

Note: The minimum number of units that resulted in complete digestion of 1 μg of substrate DNA in 16 hours is 0,25. AfeI cleaves supercoiled and linear plasmid DNA (pBR322) at a roughly equal rate. AfeI cleaves Lambda DNA/BamHI digest at a rate 3-4 times higher than plasmid DNA.

CERTIFICATE OF ANALYSIS