AfeI

NEW ENGLAND BioLabs Inc.
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R0652S 004120914091

R0652S

200 units **10,000 U/ml** **Lot: 0041209**
RECOMBINANT **Store at –20°C** **Exp: 9/14**

Recognition Site:

5′... A G C▼G C T ... 3′
3′... T C G▲C G A ... 5′

Source: An *E.coli* strain that carries the cloned AfeI gene from *Alcaligenes faecalis* T2774 (S.K. Degtyarev)

Supplied in: 300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Reagents Supplied with Enzyme:
10X NEBuffer 4

Reaction Conditions: 1X NEBuffer 4
Incubate at 37°C.

1X NEBuffer 4:
20 mM Tris-acetate
10 mM magnesium acetate
50 mM potassium acetate
1 mM DTT
pH 7.9 @ 25°C

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

Diluent Compatibility: Diluent Buffer B
300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Control Assays

Ligation: After a 10-fold overdigestion with AfeI, > 95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5′ termini concentration of 1-2 µM) at 16°C. Of these ligated fragments, > 95% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of pXba DNA and 10 units of enzyme incubated for 16 hours at 37°C resulted in a DNA pattern free of detectable nuclease degradation as determined by gel electrophoresis.

Exonuclease Activity: Incubation of a 50 µl reaction containing 100 units of AfeI with 1 µg of a mixture of single and double-stranded [^{3}H] *E.coli* DNA (200,000 cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of a 50 µl reaction containing 10 units of AfeI with 1 µg of ϕX174 RF I DNA for 4 hours at 37°C resulted in < 20% conversion to RF II as determined by agarose gel electrophoresis.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	25%
NEBuffer 2	50%
NEBuffer 3	25%
NEBuffer 4	**100%**

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

Survival in a Reaction: Intermediate activity. Suitable for extended digestion, but < 8 hours.

Heat Inactivation: 65°C for 20 minutes.

Notes: AfeI is an isoschizomer of Eco47III.

Cleavage of mammalian genomic DNA is blocked by CpG methylation.

Conditions of low ionic strength, high enzyme concentration, glycerol concentration > 5% or pH > 8.0 may result in star activity.

CERTIFICATE OF ANALYSIS

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