AflII

1-800-632-7799
info@neb.com
www.neb.com

R0520S 042121014101

R0520S

2,000 units **20,000 U/ml** **Lot: 0421210**
RECOMBINANT **Store at –20°C** **Exp: 10/14**

Recognition Site:

5′... C▼TTAAG ...3′
3′... GAATT▲C ...5′

Source: An *E. coli* strain that carries the cloned AflII gene from *Anabaena flos-aquae* (CCAP 1403/13f)

New Reaction Buffer

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.5 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 4, 100X BSA.

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 µg/ml BSA. Incubate at 37°C.

1X NEBuffer 4:
50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium 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 ϕX174 RF I DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

Diluent Compatibility: Diluent Buffer A
50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Control Assays

Ligation: After 5-fold overdigestion with AflII , approximately 50% 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 DNA and 300 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme.

Exonuclease Activity: Incubation of 200 units of enzyme with 1 µg sonicated ^{3}H DNA (10^{5} cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.4% radioactivity.

Endonuclease Activity: Incubation of 100 units of enzyme with 1 µg pBR322 DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 10% conversion from supercoiled to RFII.

Blue/White Screening Assay: This enzyme has been tested to determine the integrity of the DNA ends produced after digestion with an excess of enzyme. An appropriate vector is digested at a unique site within *lacZ*$^{\alpha}$ gene with a 10-fold excess of enzyme, ligated, transformed and plated on XGal/IPTG/Amp plates. Successful expression of β-galactosidase is a function of how intact its gene remains after cloning, an intact gene gives rise to a blue colony, an interrupted gene (i.e. degraded DNA end) gives rise to a white colony. Enzymes must produce fewer than 3% white colonies to be Blue/White Certified.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	50%
NEBuffer 2	100%
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.

(See other side)

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

Survival in a Reaction: Suitable for an extended or overnight digestion. Enzyme is active > 8 hours.

Heat Inactivation: 100 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Plasmid Cleavage: Number of units required to cleave 1 µg of supercoiled plasmid DNA in one hour: 2 units.

Notes: Not sensitive to *dam, dcm* or mammalian CpG methylation. Less than 50% of AflII fragments ligate in a standard 20 µl reaction containing 100–500 units of T4 DNA Ligase.

= Time-Saver™ Qualified (See www.neb.com for details).

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