FokI

NEW ENGLAND BioLabs Inc.
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R0109S 047120714071

R0109S

1,000 units **4,000 U/ml** **Lot: 0471207**
RECOMBINANT **Store at –20°C** **Exp: 7/14**

Recognition Site:

5′... G G A T G $(N)_9$▼... 3′
3′... C C T A C $(N)_{13}$▲... 5′

Source: An *E. coli* strain that carries the cloned FokI gene from *Flavobacterium okeanokoites* (IF0 12536)

Supplied in: 50 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA, 50% glycerol and 0.1% tween 20.

Reagents Supplied with Enzyme:
10X NEBuffer 4.

Reaction Conditions: 1X NEBuffer 4. 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 λ 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 10-fold overdigestion with FokI, > 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 approximately 75% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of 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 with 1 unit of enzyme.

Exonuclease Activity: Incubation of 6 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 < 0.3% radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	100%
NEBuffer 2	100%
NEBuffer 3	75%
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: A minimum of 1.00 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 5 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Notes: FokI can cleave between virtually any two nucleotides by constructing a complementary oligonucleotide to the sequence to be cleaved (Szybalski, W. (1985) *Gene* 40, 169–173, Podhajska, A. and Szybalski, W. (1985) *Gene* 40, 175–182). Overdigestions of > 5 units of FokI per µg of DNA and incubation times > 2 hours are not recommended.

Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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

U.S. Patent No. 4,999,294

CERTIFICATE OF ANALYSIS

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Enzyme Properties
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NEBuffer 1 100%
NEBuffer 2 100%
NEBuffer 3 75%
NEBuffer 4 100%
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