

Sfcl



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R0561S 027120714071

R0561S



200 units Lot: 0271207 Exp: 7/14
10,000 U/ml Store at -20°C

Recognition Site:

5'...**C**T R Y A G...3'
3'...G A Y R T**C**...5'

Single Letter Code: R = A or G, Y = C or T

Source: An *E. coli* strain that carries the cloned Sfcl gene from *Streptococcus faecium* (A. Meloni)

4X More Units

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4X More Units

Supplied in: 300 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 500 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 λ 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).

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Quality Control Assays

Ligation: After 2-fold overdigestion with Sfcl, > 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 DNA and 50 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 50 units of enzyme with 1 µg sonicated ³H DNA (10⁵ cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 1.0% radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1 75%
NEBuffer 2 50%
NEBuffer 3 10%
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.

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Survival in a Reaction: A minimum of 1.0 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

Plasmid Cleavage: Number of units required to cleave 1 µg of plasmid DNA in one hour: pBR322 = 2 units, pUC19 = 4 units.

Notes: Sfcl concentrations of < 1 unit/µg DNA in a reaction are **not** recommended. Sfcl is active for one hour in a reaction at 37°C, after one hour no further digestion takes place. The stability is greatly enhanced by incubations at 25°C. Although the enzyme is only 25% as active at this temperature, we recommend an incubation temperature of 25°C for 4–16 hours when digesting supercoiled plasmids. At 25°C, 0.1 unit will digest 1 µg of substrate DNA in 16 hours.

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

CERTIFICATE OF ANALYSIS

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