StyI-HF™

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

R3500S 00112081408 1

R3500S

3,000 units **20,000 U/ml** **Lot: 0011208**

RECOMBINANT **Store at –20°C** **Exp: 8/14**

Recognition Site:

5′. . . C▼C W W G G . . . 3′
3′. . . G G W W C▲C . . . 5′

Single Letter Code: W = A or T

Note: StyI-HF™ has the same specificity as StyI (NEB #R0500), but it has been engineered for reduced star activity.

Source: An *E. coli* strain that carries the cloned and modified StyI gene from *Salmonella typhi* (E.K. Anderson)

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

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 at 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 Controls

Ligation: After 50-fold overdigestion with StyI-HF, > 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 frament, > 95% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 100 units of enzyme incubated for 16 hours resulted in no degradation of the DNA bands due to nonspecific nucleases.

Exonuclease Activity: Incubation of 100 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 < 0.05% radioactivity.

Endonuclease Activity: Incubation of 20 units of enzyme with 1 µg ϕX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 10% conversion to RF II.

Enzyme Properties

Activity in NEBuffers

NEBuffer 1	25%
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.

Survival in a Reaction: A minimum of 0.25 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 10 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

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

New icons (see www.neb.com for details)
= Time-Saver™ Qualified
= indicates that the enzyme has been engineered
= indicates that the enzyme has reduced star activity

CERTIFICATE OF ANALYSIS

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