

BsgI



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



R0559S 050120814081

R0559S

50 units **Lot: 0501208** **Exp: 8/14**

5,000 U/ml **Store at -20°C**

Recognition Site:

5'... GTGCAG(N)₁₆... 3'
3'... CACGTC(N)₁₄... 5'

Source: *Bacillus sphaericus* B922 (H. Kong)

Supplied in: 200 mM KCl, 10 mM Tris-HCl
(pH 7.5), 0.1 mM EDTA, 1 mM DTT,
0.05% Triton X-100 and 50% glycerol.

Reagents Supplied with Enzyme:

10X NEBuffer 4, 400X S-adenosylmethionine
(32 mM).

Reaction Conditions:

1X NEBuffer 4,
80 μM S-adenosylmethionine.
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 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 5-fold overdigestion with BsgI,
> 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 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 60 μl
reaction buffer released < 0.1% radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1 50%
NEBuffer 2 75%
NEBuffer 3 50%
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: 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 supercoiled plasmid DNA in one
hour: 1 unit.

Notes: BsgI requires 80 μM S-adenosyl
methionine in reaction mixture for optimal activity
(supplied with enzyme). Incubation without
S-adenosylmethionine results in 25% activity.

SAM should be kept frozen at shipping
concentration and diluted prior to each reaction.

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

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

CERTIFICATE OF ANALYSIS

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