

Bcgl



1-800-632-7799
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R0545S 027121114111

R0545S



250 units 2,000 U/ml Lot: 0271211
RECOMBINANT Store at -20°C Exp: 11/13

Recognition Site:

5'...₁₀(N)CGA(N)₆TGC(N)₁₂...3'
3'...₁₂(N)GCT(N)₆ACG(N)₁₀...5'

Source: An *E. coli* strain that carries the cloned Bcgl gene from *Bacillus coagulans* (H. Kong)

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

Reagents Supplied with Enzyme:

10X NEBuffer 3,
1600X S-adenosylmethionine (SAM) (32 mM).

Reaction Conditions:

1X NEBuffer 3.
Supplemented with 20 µM S-adenosylmethionine.
Incubate at 37°C.

1X NEBuffer 3:

100 mM NaCl
50 mM Tris-HCl
10 mM MgCl₂
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).

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Quality Control Assays

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 10 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 15 units of enzyme with 1 µg sonicated ³H DNA (10⁵ cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.2% radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1 50%
NEBuffer 2 75%
NEBuffer 3 100%
NEBuffer 4 50%

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

Notes: Bcgl cleaves DNA substrates twice to excise its recognition site generating a 32 base-pair fragment with 2-base 3' overhangs.

Requires S-adenosylmethionine for optimal activity (supplied with enzyme).

S-adenosylmethionine is stored at -20°C as 32 mM solution dissolved in sulfuric acid (0.005 M) and 10% ethanol. SAM in this solution stored under ideal conditions remains active for up to 6 months. SAM is unstable at (pH 7.5), 37°C, and should be replenished for reactions incubated longer than 4 hours.

(See other side)

CERTIFICATE OF ANALYSIS

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(See other side)

CERTIFICATE OF ANALYSIS

Many problems in achieving complete digestion can be alleviated by using fresh SAM.

Impaired by overlapping *dam* methylation. Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation.

Companion Products:

dam⁻/*dcm*⁻ Competent *E. coli*

#C2925H 20 transformation reactions

#C2925I 24 transformation reactions

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