

Cac8I



1-800-632-7799
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R0579S 022121114111

R0579S



100 units Lot: 0221211 Exp: 11/14
5,000 U/ml Store at -20°C

Recognition Site:

5'...GCN ∇ NGC...3'
3'...CGN \blacktriangle CG...5'

Source: *Clostridium acetobutylicum* ABKn8
(G. Reysett)

New Reaction Buffer

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Source: *Clostridium acetobutylicum* ABKn8
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New Reaction Buffer

Supplied in: 150 mM KCl, 10 mM Tris-HCl
(pH 7.5), 0.1 mM EDTA, 1 mM DTT,
0.1% Triton X-100 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 dithiothreitol
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount
of enzyme required to digest 1 μ g λ DNA in
1 hour at 37°C in a total reaction volume of 50 μ l.

Diluent Compatibility: Diluent Buffer B
300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA,
1 mM DTT, 500 μ g/ml BSA and 50% glycerol
(pH 7.4 @ 25°C).

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(pH 7.4 @ 25°C).

Quality Control Assays

Ligation: After 5-fold overdigestion with Cac8I,
> 95% of the DNA fragments can be ligated with
T4 DNA Ligase (at a 5' termini concentration of
1–2 μ M) at 16°C. Of these ligated fragments,
> 95% can be recut.

16-Hour Incubation: A 50 μ l reaction containing
1 μ g of DNA and 50 units of enzyme incubated
for 16 hours resulted in the same pattern of DNA
bands as a reaction produced in one hour with
1 unit of enzyme.

Exonuclease Activity: Incubation of 60 units
of enzyme with 1 μ g sonicated 3 H DNA
(10⁵ cpm/ μ g) for 4 hours at 37°C in 50 μ l reaction
buffer released < 0.12% radioactivity.

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Enzyme Properties

Activity in NEBuffers:
NEBuffer 1 50%
NEBuffer 2 75%
NEBuffer 3 100%
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 μ g of substrate DNA in
16 hours.

Heat Inactivation: 30 units of enzyme were
inactivated by incubation at 65°C for 20 minutes.

Note: Cleavage of mammalian genomic DNA is
blocked by some combinations of overlapping
CpG methylation.).

CERTIFICATE OF ANALYSIS

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