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 300 units
 3,000 U/ml
 Lot: 0101210

 RECOMBINANT Store at -20°C
 Exp: 10/14

Recognition Site:

5′... A ACGTT... 3′ 3′... TTGCAA... 5′

Source: An *E. coli* strain that carries the cloned Acll gene from *Acinetobacter calcoaceticus* M4 (S.K. Degtyarev)





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Recognition Site:

5′... A Å C G T T ... 3′ 3′... T T G C A A ... 5′

Source: An *E. coli* strain that carries the cloned Acll gene from *Acinetobacter calcoaceticus* M4 (S.K. Degtyarev) Supplied in: 100 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme: 10X NEBuffer 4, 100X BSA.

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 μ g/ml BSA. 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 of λ DNA in 1 hour at 37°C in a total reaction volume of 50 μ l.

Diluent Compatibility: Diluent Buffer B 300 mM NaCI, 10 mM Tris-HCI, 0.1 mM EDTA, 1 mM dithiothreitol, 0.1 mM EDTA, 500 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Supplied in: 100 mM KCl, 10 mM Tris-HCl

200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:

Reaction Conditions: 1X NEBuffer 4.

supplemented with 100 µg/ml BSA.

10X NEBuffer 4, 100X BSA.

50 mM potassium acetate 20 mM Tris-acetate

10 mM magnesium acetate

Incubate at 37°C.

1 mM dithiothreitol

pH 7.9 @ 25°C

volume of 50 µl.

1X NEBuffer 4:

(pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol,

Quality Control Assays

Ligation: After 10-fold overdigestion with AcII, > 95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5' termini concentration of $1-2 \mu$ M) at 16°C. Of these ligated fragments, > 95% can be recut.

16-Hour Incubation: A 50 μ I reaction containing 1 μ g of DNA and 30 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 μ g sonicated ³H DNA (10⁵ cpm/ μ g) for 4 hours at 37°C in 50 μ l reaction buffer released < 0.2% radioactivity.

Enzyme Properties

Activity in NEBuffers: NEBuffer 1 10%

NEBuffer 2 10% NEBuffer 3 0% 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: No

Notes: Acll is an isoschizomer of Psp14061.

Cleavage of mammalian genomic DNA is blocked by CpG methylation.

Image: Saver[™] Qualified (See www.neb.com for details).

CERTIFICATE OF ANALYSIS

Quality Control Assays

Ligation: After 10-fold overdigestion with AcIl, > 95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5' termini concentration of $1-2 \ \mu$ M) at 16°C. Of these ligated fragments, > 95% can be recut.

16-Hour Incubation: A 50 μ I reaction containing 1 μ g of DNA and 30 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 μ g sonicated ³H DNA (10⁵ cpm/ μ g) for 4 hours at 37°C in 50 μ l reaction buffer released < 0.2% radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	10%
NEBuffer 2	10%
	00/

NEBuffer 3 0%

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: No

Notes: Acll is an isoschizomer of Psp1406I.

Cleavage of mammalian genomic DNA is blocked by CpG methylation.

Image: Saver[™] Qualified (See www.neb.com for details).

Unit Definition: One unit is defined as the

 λ DNA in 1 hour at 37°C in a total reaction

amount of enzyme required to digest 1 µg of