AcuI

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

R0641S 008120814081

R0641S

300 units **5,000 U/ml** **Lot: 0081208**

RECOMBINANT Store at –20°C Exp: 8/14

Recognition Site:

5′... C T G A A G $(N)_{16}$ ▼... 3′
3′... G A C T T C $(N)_{14}$ ▲... 5′

Source: An *E. coli* strain that carries the cloned AcuI gene from *Acinetobacter calcoaceticus*

Supplied in: 100 mM NaCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol.

More Units, New Reaction Buffer

Reagents Supplied with Enzyme:
10X NEBuffer 4, 800X S-adenosylmethionine (32 mM).

Reaction Conditions: 1X NEBuffer 4, supplemented with 40 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 reaction 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 10-fold overdigestion with AcuI, > 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, > 95% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 5 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 [^{3}H] DNA (10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.2% 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 < 20% conversion to RF II.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	50%
NEBuffer 2	100%
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: A minimum of 1 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 50 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Notes: 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.

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

Conditions of high enzyme concentration, glycerol concentration > 5% or pH > 8.0 may result in star activity.

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

CERTIFICATE OF ANALYSIS

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