Acc65I

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

R0599S 015121014101

R0599S

BSA 37° Yes dcm NEB3

2,000 units **10,000 U/ml** **Lot: 0151210**
RECOMBINANT **Store at –20°C** **Exp: 10/14**

Recognition Site:

5′...G▼GTACC...3′
3′...CCATG▲G...5′

Source: An *E. coli* strain that carries the cloned Acc65I gene from *Acinetobacter calcoaceticus* 65 (S.K. Degtyarev)

Supplied in: 100 mM NaCl, 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 3, 100X BSA

Reaction Conditions: 1X NEBuffer 3, supplemented with 100 µg/ml BSA. Incubate at 37°C.

1X NEBuffer 3:
100 mM NaCl
50 mM Tris-HCl
10 mM $MgCl_2$
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 pBC4 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).

Quality Control Assays

Ligation: After 20-fold overdigestion with Acc65I, > 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 100 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 300 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 < 0.1% radioactivity.

Endonuclease Activity: Incubation of 50 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.

Blue/White Screening Assay: This enzyme has been tested to determine the integrity of the DNA ends produced after digestion with an excess of enzyme. An appropriate vector is digested at a unique site within *lacZ*^{a} gene with a 10-fold excess of enzyme, ligated, transformed and plated on XGal/IPTG/Amp plates. Successful expression of β-galactosidase is a function of how intact its gene remains after cloning, an intact gene gives rise to a blue colony, an interrupted gene (i.e. degraded DNA end) gives rise to a white colony. Enzymes must produce fewer than 3% white colonies to be Blue/White Certified.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	10%
NEBuffer 2	75%
NEBuffer 3	**100%**
NEBuffer 4	25%

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

(See other side)

CERTIFICATE OF ANALYSIS

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(See other side)
CERTIFICATE OF ANALYSIS

Survival in a Reaction: Intermediate activity. Suitable for extended digestion, but < 8 hours.

Heat Inactivation: 50 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: Acc65I is an neoschizomer of KpnI.

Blocked by some combinations of overlapping *dcm* 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

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

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