AvrII

1-800-632-7799
info@neb.com
www.neb.com

R0174S 045121014101

R0174S

100 units **4,000 U/ml** **Lot: 0441210**
RECOMBINANT **Store at –20°C** **Exp: 10/14**

Recognition Site:

5′. . . C▼C T A G G . . . 3′
3′. . . G G A T C▲C . . . 5′

Source: An *E. coli* strain that carries the cloned AvrII gene from *Anabaena variabilis* UW (E. Rosenvold)

New Reaction Buffer

Supplied in: 250 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 500 µg/ml BSA 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 µg of λ DNA (HindIII digest) 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 dithiothreitol, 500 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Control Assays

Ligation: After 100-fold overdigestion with AvrII, > 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 200 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 80 units of enzyme with 1 µg ϕX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in 10% conversion to RF II.

Blue/White Screening Assay: An appropriate vector is digested at a unique site within the *lacZ*$^{\alpha}$ gene with a 10-fold excess of enzyme. The vector DNA is then ligated, transformed, and plated onto 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, removal of even a single base gives rise to a white colony. Enzyme preparations must produce fewer than 3% white colonies to be Blue/White certified.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	100%
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 0.13 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 80°C for 20 minutes.

Plasmid Cleavage: Number of units required to cleave 1 µg of supercoiled plasmid DNA in one hour: 1 unit.

(See other side)

CERTIFICATE OF ANALYSIS

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Notes: Cleaves to produce a 5´ CTAG extension which can be efficiently ligated to DNA fragments generated by NheI, SpeI or XbaI.

Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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

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