AgeI

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

R0552S 043120914091

R0552S

NEB1 37° Yes

300 units **5,000 U/ml** **Lot: 0431209**
RECOMBINANT **Store at –20°C** **Exp: 9/14**

Recognition Site:

5′. . . A▼CCGGT . . . 3′
3′. . . TGGCC▲A . . . 5′

Source: An *E. coli* strain that carries the cloned AgeI gene from *Ruegeria gelatinovora* (ATCC 25655)

More Units

Supplied in: 250 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 0.15% Triton X-100, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 1.

Reaction Conditions: 1X NEBuffer 1. Incubate at 37°C.

1X NEBuffer 1:
10 mM Bis Tris Propane-HCl
10 mM $MgCl_2$
1 mM DTT
pH 7.0 @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 of 50 µl.

Diluent Compatibility: Diluent Buffer C
250 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 0.15% Triton X-100 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

Quality Control Assays

Ligation: After 10-fold overdigestion with AgeI, > 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 50 units of enzyme incubated for 16 hours resulted in no degradation of the DNA bands due to nonspecific nucleases. However, fragments produced by noncanonical cleavage due to star activity may be observed with 5 units of enzyme in similar conditions.

Exonuclease Activity: Incubation of 50 units of enzyme with 1 µg sonicated ^3H DNA (10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

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*$^{\alpha}$ gene with a 10-fold excess of enzyme, ligated, transformed and plated on XGalIPTG/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	**100%**
NEBuffer 2	50%
NEBuffer 3	10%
NEBuffer 4	75%

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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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.

Note: Cleavage of mammalian genomic DNA is blocked by CpG methylation.

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

Companion Products:

AgeI-HF™
#R3552S 300 units
#R3552L 1,500 units

AgeI-HF™ RE-Mix™
#R5552S 25 reactions

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

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