AgeI-HF™

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

R3552S 003120814081

R3552S

300 units **20,000 U/ml** **Lot: 0031208**
RECOMBINANT **Store at –20°C** **Exp: 8/14**

Recognition Site:

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

Note: AgeI-HF™ has the same specificity as AgeI (NEB #R0552), but it has been engineered for reduced star activity.

More Units

Source: An *E. coli* strain that carries the cloned and modified (R139A) AgeI gene from *Ruegeria gelatinovora* (ATCC 25655)

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50% glycerol.

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

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 µg/ml BSA. 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 at 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 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 Controls

Ligation: After 50-fold overdigestion with AgeI-HF, > 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.

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	**100**%

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

AgeI-HF™
1-800-632-7799
info@neb.com
www.neb.com
R3552S 003120814081
R3552S
300 units 20,000 U/ml Lot: 0031208
RECOMBINANT Store at –20°C Exp: 8/14
Recognition Site:
5′. . . A▼C C G G T . . . 3′
3′. . . T G G C C▲A . . . 5′
Note: AgeI-HF™ has the same specificity as AgeI (NEB #R0552), but it has been engineered for reduced star activity.
More Units
Source: An E. coli strain that carries the cloned and modified (R139A) AgeI gene from Ruegeria gelatinovora (ATCC 25655)
Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50% glycerol.
Reagents Supplied with Enzyme:
10X NEBuffer 4, 100X BSA.
Reaction Conditions: 1X NEBuffer 4, supplemented with 100 µg/ml BSA. 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 at 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 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 Controls
Ligation: After 50-fold overdigestion with AgeI-HF, > 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 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 lacZα 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 4 100%
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

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

Heat Inactivation: 100 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.

Companion Products:

AgeI
#R0552S 300 units
#R0552L 1,500 units

AgeI-HF™ RE-Mix™
#R5552S 25 reactions

New icons (see www.neb.com for details)
= Time-Saver™ Qualified
= indicates that the enzyme has been engineered
= indicates that the enzyme has reduced star activity

U.S. Patent No. 5,885,818

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

Heat Inactivation: 100 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.

Companion Products:

AgeI
#R0552S 300 units
#R0552L 1,500 units

AgeI-HF™ RE-Mix™
#R5552S 25 reactions

New icons (see www.neb.com for details)
= Time-Saver™ Qualified
= indicates that the enzyme has been engineered
= indicates that the enzyme has reduced star activity

U.S. Patent No. 5,885,818

Page 2 (R3552)