

AgeI-HF™ RE-Mix®



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



R5552S 002120113011

R5552S



25 reactions Lot: 0021201 Exp: 1/13

RECOMBINANT Store at -20°C

Recognition Site:

5'... A[▼]CCGGT... 3'
3'... TGGCC[▲]A... 5'

Description: AgeI-HF RE-Mix is a ready to use 10X master mix combining the restriction enzyme, AgeI-HF (NEB #R3552), NEBuffer, BSA and loading dye in a single tube. Simply add DNA and water and incubate for 15 minutes to perform the restriction digest.

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

Reaction Conditions: 1X AgeI-HF RE-Mix and DNA in a total reaction volume of 20 µl. Incubate at 37°C for 15 minutes.

One reaction is defined as the amount of RE-Mix that will digest 1 µg of DNA in 15 minutes at 37°C in a total reaction volume of 20 µl.

Protocol – Standard Digest:

1. Dilute up to 1 µg DNA to 18 µl with dH₂O
2. Add 2 µl of 10X RE-Mix
3. Incubate for 15 minutes at 37°C
4. Analyze by agarose gel electrophoresis

Protocol – Double Digest:

1. Dilute up to 1 µg DNA to 36 µl with dH₂O
2. Add 2 µl of each 10X RE-Mix
3. Incubate for 15 minutes at 37°C
4. Analyze by agarose gel electrophoresis

Notes: Alternatively, double digest can be carried out in a 20 µl volume using 1 µl of each RE-Mix and a minimum incubation time of 30 minutes.

Quality Control Assays

16-Hour Incubation: A 20 µl reaction containing 1 µg of DNA and 1X AgeI-HF RE-Mix incubated for 16 hours at 37°C resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Exonuclease Activity: Incubation of a 20 µl reaction containing 1X AgeI-HF RE-Mix with 1 µg of a mixture of single and double-stranded [³H] *E. coli* DNA (10⁵ cpm/µg) incubated for 4 hours at 37°C releases < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of a 20 µl reaction containing 1X AgeI-HF RE-Mix with 1 µg of φX174 RF I DNA incubated for 4 hours at 37°C resulted in < 10% conversion to RF II as determined by agarose gel electrophoresis.

Ligation and Re-cutting: After overdigestion with 1X AgeI-HF RE-Mix, > 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 with AgeI-HF.

Enzyme Properties

Heat Inactivation: 1X RE-Mix is inactivated by incubation at 65°C for 20 minutes.

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

Companion Products Sold Separately:

AgeI
#R0552S 250 units
#R0552L 1,250 units

AgeI-HF
#R3552S 250 units
#R3552L 1,250 units

Gel Loading Dye, Blue
#B7021S

RE-MIX® is a registered trademark of New England Biolabs, Inc.
HF™ is a trademark of New England Biolabs, Inc.

Relevant patent applications include: US2009-0029376, EP2392654, CN101802183A, IN 7681/CHENP/2009 and JP 2010-516300.

