NEW ENGLAND BioLabs Inc.
be INSPIRED drive DISCOVERY stay GENUINE
240 County Road
Ipswich, MA 01938-2723
Tel 978-927-5054
Fax 978-921-1350
www.neb.com
info@neb.com

New England Biolabs
Certificate of Analysis

Product Name:	*AgeI-HF®*
Catalog Number:	*R3552S*
Concentration:	*20,000 U/ml*
Unit Definition:	*One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in rCutSmart Buffer in 1 hour at 37°C in a total reaction of 50 µl.*
Packaging Lot Number:	*10145787*
Expiration Date:	*04/2024*
Storage Temperature:	*-20°C*
Storage Conditions:	*10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml rAlbumin (pH 7.4 @ 25°C)*
Specification Version:	*PS-R3552S/L v2.0*

AgeI-HF® Component List

NEB Part Number	Component Description	Lot Number	Individual QC Result
R3552SVIAL	AgeI-HF®	10145773	**Pass**
B7024AVIAL	Gel Loading Dye, Purple (6X)	10143287	**Pass**
B6004SVIAL	rCutSmart™ Buffer	10144738	**Pass**

Assay Name/Specification	Lot # 10145787
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [^{3}H] E. coli DNA and a minimum of 200 units of AgeI-HF® incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	**Pass**
Blue-White Screening (Terminal Integrity) A sample of LITMUS28i vector linearized with a 10-fold excess of AgeI-HF®, religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in <1% white colonies.	**Pass**
qPCR DNA Contamination (E. coli Genomic) A minimum of 20 units of AgeI-HF® is screened for the presence of E. coli genomic DNA using SYBR® Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is ≤ 1 E. coli genome.	**Pass**

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Assay Name/Specification	Lot # 10145787
Protein Purity Assay (SDS-PAGE) AgeI-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 100 units of AgeI-HF® incubated for 16 hours at 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	**Pass**
Ligation and Recutting (Terminal Integrity) After a 20-fold over-digestion of Lambda DNA with AgeI-HF®, >95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16ºC. Of these ligated fragments, >95% can be recut with AgeI-HF®.	**Pass**
Functional Testing (15 minute Digest) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and 1 µl of AgeI-HF® incubated for 15 minutes at 37ºC results in complete digestion as determined by agarose gel electrophoresis.	**Pass**

This product has been tested and shown to be in compliance with all specifications.

Penghua Zhang
Production Scientist
09 May 2022

Michael Tonello
Packaging Quality Control Inspector
09 May 2022