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:	*AfeI*
Catalog Number:	*R0652S*
Concentration:	*10,000 U/ml*
Unit Definition:	*One unit is defined as the amount of enzyme required to digest 1 µg of pXba DNA in rCutSmart Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.*
Packaging Lot Number:	*10258556*
Expiration Date:	*09/2026*
Storage Temperature:	*-20°C*
Storage Conditions:	*10 mM Tris-HCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 500 µg/ml rAlbumin (pH 7.4 @ 25°C)*
Specification Version:	*PS-R0652S/L v2.0*

AfeI Component List

NEB Part Number	Component Description	Lot Number	Individual QC Result
R0652SVIAL	AfeI	10254294	**Pass**
B7024AVIAL	Gel Loading Dye, Purple (6X)	10236426	**Pass**
B6004SVIAL	rCutSmart™ Buffer	10249065	**Pass**

Assay Name/Specification	Lot # 10258556
Endonuclease Activity (Nicking) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 10 units of AfeI incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.	**Pass**
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 100 units of AfeI incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	**Pass**
Ligation and Recutting (Terminal Integrity) After a 10-fold over-digestion of pXba DNA with AfeI, >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 AfeI.	**Pass**
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of pXba DNA and a minimum of	**Pass**

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Assay Name/Specification	Lot # 10258556
10 units of AfeI incubated for 16 hours at 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	
qPCR DNA Contamination (E. coli Genomic) A minimum of 10 units of AfeI 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**

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

Nancy Considine
Production Scientist
09 Sep 2024

Michael Tonello
Packaging Quality Control Inspector
11 Sep 2024