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New England Biolabs Certificate of Analysis

Product Name: Xbal
Catalog Number: R0145L
Concentration: 20,000 U/ml

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg

of Lambda DNA (dam-/HindIII digest) in rCutSmart Buffer in 1 hour at

37°C in a total reaction volume of 50 μl.

Packaging Lot Number: 10165078
Expiration Date: 09/2024
Storage Temperature: -20°C

Storage Conditions: 10 mM Tris-HCl, 50 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200

 $\mu g/ml$ rAlbumin, (pH 7.4 @ 25°C)

Specification Version: PS-R0145S/L/V v3.0

Xbal Component List				
NEB Part Number	Component Description	Lot Number	Individual QC Result	
R0145LVIAL	Xbal	10165077	Pass	
B7024AVIAL	Gel Loading Dye, Purple (6X)	10165690	Pass	
B6004SVIAL	rCutSmart™ Buffer	10162782	Pass	

Assay Name/Specification	Lot # 10165078
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda-HindIII dam- DNA and a minimum of 200 units of Xbal incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass
RNase Activity (Extended Digestion) A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 20 units of Xbal is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.	Pass
qPCR DNA Contamination (E. coli Genomic) A minimum of 20 units of Xbal 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 # 10165078
Protein Purity Assay (SDS-PAGE) Xbal is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.	Pass
Blue-White Screening (Terminal Integrity) A sample of pUC19 vector linearized with a 10-fold excess of Xbal, religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in <1% white colonies.	Pass
Endonuclease Activity (Nicking) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 100 units of Xbal incubated for 4 hours at 37°C results in <10% 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 [³H] E. coli DNA and a minimum of 200 units of Xbal 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 pBC4XS DNA with Xbal, >95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, >95% can be recut with Xbal.	Pass
Functional Testing (15 minute Digest) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda-HindIII dam- DNA and 1 µl of Xbal incubated for 15 minutes at 37°C 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.

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.



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YunJie Sun Production Scientist 20 Sep 2022

Michael Tonello

Packaging Quality Control Inspector

13 Oct 2022

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