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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: 10190446
Expiration Date: 03/2025
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	10183034	Pass	
B7024AVIAL	Gel Loading Dye, Purple (6X)	10186770	Pass	
B6004SVIAL	rCutSmart™ Buffer	10184700	Pass	

Assay Name/Specification	Lot # 10190446
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	Pass
<1% white colonies.	
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
Functional Testing (15 minute Digest) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda-HindIII dam- DNA and	Pass



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Assay Name/Specification	Lot # 10190446
1 μl of Xbal incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.	
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
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
Protein Purity Assay (SDS-PAGE) Xbal is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.	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

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

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YunJie Sun \
Production Scientist 24 Mar 2023

Josh Hersey Packaging Quality Control Inspector 02 Jun 2023

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