

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: Xhol
Catalog Number: R0146S
Concentration: 20,000 U/ml

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

of Lambda DNA (HindIII digest) fragments in rCutSmart Buffer in 1

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

Packaging Lot Number: 10202022
Expiration Date: 05/2025
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-R0146S/L/E v3.0

Xhol Component List				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
R0146SVIAL	Xhol	10190225	Pass	
B7024AVIAL	Gel Loading Dye, Purple (6X)	10193044	Pass	
B6004SVIAL	rCutSmart™ Buffer	10196037	Pass	

Assay Name/Specification	Lot # 10202022
Blue-White Screening (Terminal Integrity) A sample of Litmus 28i vector linearized with a 10-fold excess of Xhol, religated and transformed into an E. coli strain expressing the LacZ beta fragment gene	Pass
results in <1% white colonies.	
Endonuclease Activity (Nicking) A 50 μl reaction in rCutSmart™ Buffer containing 1 μg of supercoiled pBR322 DNA and	Pass
a minimum of 100 units of XhoI incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	
Exonuclease Activity (Radioactivity Release)	Pass
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 100 units of Xhol incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	
Functional Testing (15 minute Digest)	Pass
A 50 μl reaction in rCutSmart™ Buffer containing 1 μg of Lambda-HindIII DNA and 1 μl	



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Assay Name/Specification	Lot # 10202022
of Xhol 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 pXba DNA with XhoI, >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 XhoI.	Pass
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda-HindIII DNA and a minimum of 100 units of Xhol 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) Xhol is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.	Pass
qPCR DNA Contamination (E. coli Genomic) A minimum of 20 units of Xhol 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 V Production Scientist 09 May 2023 Michael Tonello

Packaging Quality Control Inspector

09 Aug 2023



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