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New England Biolabs Certificate of Analysis

Product Name: Xbal
Catalog Number: R0145M
Concentration: 100,000 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: 10146949
Expiration Date: 04/2024
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-R0145T/M v3.0

| Xbal Component List | | | | |
|------------------------|------------------------------|------------|----------------------|--|
| NEB Part Number | Component Description | Lot Number | Individual QC Result | |
| R0145MVIAL | Xbal | 10146948 | Pass | |
| B7024AVIAL | Gel Loading Dye, Purple (6X) | 10144740 | Pass | |
| B6004SVIAL | rCutSmart™ Buffer | 10143289 | Pass | |

| Assay Name/Specification | Lot # 10146949 |
|---|----------------|
| 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 |
| 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 |
| RNase Activity (Extended Digestion) | Pass |



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| Assay Name/Specification | Lot # 10146949 |
|---|----------------|
| 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. | |
| 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 |
| 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 |
| 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 |
| 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 |
| Protein Purity Assay (SDS-PAGE) Xbal is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection. | Pass |

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

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Penghaa Zhang Production Scientist 25 Apr 2022

Michael Tonello

Packaging Quality Control Inspector

25 Apr 2022