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## New England Biolabs Certificate of Analysis

Product Name: Xmal
Catalog Number: R0180M
Concentration: 50,000 U/ml

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

pXba in rCutSmart Buffer in 1 hour at 37°C in a total reaction

volume of 50 μl.

Packaging Lot Number: 10204867
Expiration Date: 08/2025
Storage Temperature: -20°C

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

 $\mu g/ml$  rAlbumin (pH 7.4 @ 25°C)

Specification Version: PS-R0180M v2.0

| Xmal Component List    |                              |            |                      |  |
|------------------------|------------------------------|------------|----------------------|--|
| <b>NEB Part Number</b> | <b>Component Description</b> | Lot Number | Individual QC Result |  |
| R0180MVIAL             | Xmal                         | 10202624   | Pass                 |  |
| B7024AVIAL             | Gel Loading Dye, Purple (6X) | 10198639   | Pass                 |  |
| B6004SVIAL             | rCutSmart™ Buffer            | 10198645   | Pass                 |  |

| Assay Name/Specification  | Lot # 10204867 |
|---|----------------|
| Endonuclease Activity (Nicking) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 50 units of Xmal incubated for 4 hours at 37°C results in <10%                                      | Pass           |
| 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 50 units of Xmal incubated for 4 hours at 37°C releases <0.2% of the total radioactivity.         |                |
| Functional Testing (15 minute Digest) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of pXba DNA and 1 µl of Xmal incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis. | Pass           |
| Ligation and Recutting (Terminal Integrity) After a 10-fold over-digestion of pXba DNA with Xmal, >95% of the DNA fragments can   | Pass           |



R0180M / Lot: 10204867

Page 1 of 2

| Assay Name/Specification  | Lot # 10204867 |
|---|----------------|
| be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, >95% can be recut with Xmal.   |                |
| Non-Specific DNase Activity (16 Hour) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of pXba DNA and a minimum of 10 units of Xmal 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) Xmal is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.  | Pass           |
| qPCR DNA Contamination (E. coli Genomic)  A minimum of 10 units of Xmal 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.

YunJie Suń

Production Scientist

17 Aug 2023

Michael Tonello

Packaging Quality Control Inspector

18 Aug 2023

R0180M / Lot: 10204867

Page 2 of 2