

New England Biolabs Product Specification

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| Product Name: | <i>XmaI</i> |
| Catalog #: | R0180S/L |
| Concentration: | 10,000 units/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. |
| Shelf Life: | 24 months |
| Storage Temp: | -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-R0180S/L v2.0 |
| Effective Date: | 03 Mar 2023 |

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 50 units of XmaI 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) - 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 XmaI 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 XmaI 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 XmaI, >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 XmaI.

Protein Purity Assay (SDS-PAGE) - XmaI is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.

qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 10 units of XmaI 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.

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 XmaI incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.





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Date 03 Mar 2023

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Quality Approver

