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New England Biolabs Certificate of Analysis

Product Name: Xmnl
Catalog Number: R0194S
Concentration: 20,000 U/ml

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

of Lambda DNA in 1 hour at 37°C in a total reaction volume of 50 μl.

Packaging Lot Number: 10245390
Expiration Date: 06/2026
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-R0194S/L/V v2.0

| XmnI Component List | | | | |
|------------------------|------------------------------|------------|----------------------|--|
| NEB Part Number | Component Description | Lot Number | Individual QC Result | |
| R0194SVIAL | Xmnl | 10244001 | Pass | |
| B7024AVIAL | Gel Loading Dye, Purple (6X) | 10236422 | Pass | |
| B6004SVIAL | rCutSmart™ Buffer | 10241726 | Pass | |

| Assay Name/Specification | Lot # 10245390 |
|--|----------------|
| Blue-White Screening (Terminal Integrity) A sample of pUC19 vector linearized with a 10-fold excess of XmnI, religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in <1% white colonies. | Pass |
| Blue-White Screening (Terminal Integrity) A sample of pUC19 vector linearized with a 10-fold excess of XmnI, religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in <1% white colonies. | Pass |
| Endonuclease Activity (Nicking) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled LITMUS38i DNA and a minimum of 60 units of Xmnl incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis. | Pass |
| Endonuclease Activity (Nicking) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled LITMUS38i DNA and a minimum of 60 units of XmnI incubated for 4 hours at 37°C results in <10% | Pass |



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| Assay Name/Specification | Lot # 10245390 |
|--|----------------|
| 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 XmnI incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. | 1 455 |
| 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 100 units of XmnI 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 DNA and 1 µl of Xmnl incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis. | Pass |
| Functional Testing (15 minute Digest) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and 1 µl of Xmnl 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 20-fold over-digestion of Lambda DNA with XmnI, ~75% 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 XmnI. | Pass |
| Ligation and Recutting (Terminal Integrity) After a 20-fold over-digestion of Lambda DNA with XmnI, ~75% 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 XmnI. | Pass |
| Non-Specific DNase Activity (16 Hour) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 100 units of Xmnl incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation 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 DNA and a minimum of 100 units of Xmnl incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. | Pass |



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| Assay Name/Specification | Lot # 10245390 |
|--|----------------|
| Protein Purity Assay (SDS-PAGE) | Pass |
| XmnI is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection. | |
| | |
| Protein Purity Assay (SDS-PAGE) | Pass |
| Xmnl is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection. | |

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.

Ana Egana
Production Scientist

Production Scientist 06 Jun 2024

Michael Tonello

Packaging Quality Control Inspector

06 Jun 2024



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