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New England Biolabs Certificate of Analysis

Product Name: Acc65I
Catalog Number: R0599S
Concentration: 10,000 U/ml

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

of pBC4 DNA in 1 hour at 37°C in a total reaction volume of 50 μl.

Packaging Lot Number: 10063594
Expiration Date: 01/2022
Storage Temperature: -20°C

Storage Conditions: 100 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50%

Glycerol, 200 μg/ml BSA

Specification Version: PS-R0599S/L v1.0

| Acc65I Component List | | | | |
|------------------------|-----------------------|------------|----------------------|--|
| NEB Part Number | Component Description | Lot Number | Individual QC Result | |
| R0599SVIAL | Acc65I | 10063593 | Pass | |
| B7203SVIAL | NEBuffer™ 3.1 | 10053974 | Pass | |

| Assay Name/Specification | Lot # 10063594 |
|---|----------------|
| Blue-White Screening (Terminal Integrity) A sample of Litmus28i vector linearized with a 10-fold excess of Acc65I, 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 NEBuffer 3.1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 50 Units of Acc65l incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis. | Pass |
| Exonuclease Activity (Radioactivity Release) A 50 µl reaction in NEBuffer 3.1 containing 1 µg of a mixture of single and double-stranded [³H] E. coli DNA and a minimum of 100 units of Acc65l incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. | Pass |
| Ligation and Recutting (Terminal Integrity) After a 20-fold over-digestion of pBC4 DNA with Acc65I, >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 Acc65I. | Pass |



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| Assay Name/Specification | Lot # 10063594 |
|---|----------------|
| | _ |
| Non-Specific DNase Activity (16 Hour) | Pass |
| A 50 µl reaction in NEBuffer 3.1 containing 1 µg of pBC4 DNA and a minimum of 100 | |
| Units of Acc65I incubated for 16 hours at 37°C results in a DNA pattern free of | |
| detectable nuclease degradation as determined by agarose gel electrophoresis. | |

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

Stephanie Cornelio Production Scientist

07 Jan 2020

Jay Minichiello

Packaging Quality Control Inspector

12 Feb 2020



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