

## 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:** 10087613  
**Expiration Date:** 10/2022  
**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	10087614	Pass
B7203SVIAL	NEBuffer™ 3.1	10085493	Pass

Assay Name/Specification	Lot # 10087613
<p><b>Ligation and Recutting (Terminal Integrity)</b>            After a 20-fold over-digestion of pBC4 DNA with Acc65I, &gt;95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with Acc65I.</p>	<b>Pass</b>
<p><b>Non-Specific DNase Activity (16 Hour)</b>            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.</p>	<b>Pass</b>
<p><b>Blue-White Screening (Terminal Integrity)</b>            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 &lt;1% white colonies.</p>	<b>Pass</b>
<p><b>Endonuclease Activity (Nicking)</b>            A 50 µl reaction in NEBuffer 3.1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 50 Units of Acc65I incubated for 4 hours at 37°C results in &lt;20% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	<b>Pass</b>

Assay Name/Specification	Lot # 10087613
<p><b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in NEBuffer 3.1 containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] E. coli DNA and a minimum of 100 units of Acc65I incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p>	<p><b>Pass</b></p>

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

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Production Scientist  
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Josh Hersey  
Packaging Quality Control Inspector  
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