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## New England Biolabs Certificate of Analysis

Product Name: Acc651
Catalog #: R0599S/L
Concentration: 10,000 units/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  $50 \mu l$ .

 Lot #:
 0151511

 Assay Date:
 11/2015

 Expiration Date:
 11/2017

 Storage Temp:
 -20°C

Storage Buffer: 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
Effective Date: 27 Sep 2013

Assay Name/Specification (minimum release criteria)	Lot #0151511
<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 <i>E. coli</i> strain expressing the LacZ beta fragment gene results in <1% white colonies.	Pass
<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 <20% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
<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] <i>E. coli</i> DNA and a minimum of 100 units of Acc65I incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
<b>Ligation and Recutting (Terminal Integrity)</b> - 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
Non-Specific DNase Activity (16 Hour) - 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.	Pass

<sup>\*</sup> The BSA in this product has been granted an EDQM "Certificate of Suitability" from the European Directorate for the Quality of Medicines (# R1-CEP-2003-204-Rev00) and has been granted a USDA Certificate for Export of Bovine Blood Plasma/Serum for Manufacture into Pharmaceutical Products.

Authorized by Derek Robinson 27 Sep 2013







Inspected by Stephanie Cornelio 04 Dec 2015

Stephani Unetto