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New England Biolabs Certificate of Analysis

Product Name:	Acc65I
Catalog #:	R0599S/L
Concentration:	10,000 units/ml
Unit Definition:	One unit is defined as the amount of enzyme required to digest 1 μ g of pBC4 DNA in 1 hour at 37°C in a total reaction volume of 50 μ l.
<i>Lot</i> #:	0151407
Assay Date:	07/2014
Expiration Date:	7/2016
Storage Temp:	-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
Effective Date:	27 Sep 2013

Assay Name/Specification (minimum release criteria)	Lot #0151407
Blue-White Screening (Terminal Integrity) - 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
Endonuclease Activity (Nicking) - A 50 μ l reaction in NEBuffer 3.1 containing 1 μ g 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
Exonuclease Activity (Radioactivity Release) - A 50 μ l reaction in NEBuffer 3.1 containing 1 μ g of a mixture of single and double-stranded [³ H] <i>E. coli</i> DNA and a minimum of 100 units of Acc651 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 Acc651, >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 Acc651.	Pass
Non-Specific DNase Activity (16 Hour) - A 50 μ l reaction in NEBuffer 3.1 containing 1 μ g 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

* 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.

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Authorized by Derek Robinson 27 Sep 2013



John J. Loci

Inspected by John Greci 10 Sep 2014