

## New England Biolabs Product Specification

**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 µg of pBC4 DNA in 1 hour at 37°C in a total reaction volume of 50 µl.  
**Shelf Life:** 24 months  
**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)

**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.

**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 Acc65I incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.

**Exonuclease Activity (Radioactivity Release)** - 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 <0.1% of the total radioactivity.

**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.

**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.

\* 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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Date 27 Sep 2013

