

## New England Biolabs Product Specification

<i>Product Name:</i>	Acc65I
<i>Catalog #:</i>	R0599S/L/V
<i>Concentration:</i>	10,000 units/ml
<i>Unit Definition:</i>	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.
<i>Shelf Life:</i>	24 months
<i>Storage Temp:</i>	-20°C
<i>Storage Conditions:</i>	100 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml BSA
<i>Specification Version:</i>	PS-R0599S/L v1.0
<i>Effective Date:</i>	09/27/2013

### 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.

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