

## New England Biolabs Product Specification

<i>Product Name:</i>	<i>TspMI</i>
<i>Catalog #:</i>	<i>R0709S/V</i>
<i>Concentration:</i>	<i>5,000 units/ml</i>
<i>Unit Definition:</i>	<i>One unit is defined as the amount of enzyme required to digest 1 µg of pBC4 plasmid DNA in 1 hour at 75°C in a total reaction volume of 50 µl.</i>
<i>Shelf Life:</i>	<i>6 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Storage Conditions:</i>	<i>20 mM Tris-HCl, 300 mM NaCl, 1 mM DTT, 1 mM EDTA, 50% Glycerol, 0.10% Triton® X-100, 200 µg/ml BSA, (pH 8.0 @ 25C)</i>
<i>Specification Version:</i>	<i>PS-R0709S/V v2.0</i>
<i>Effective Date:</i>	<i>25 Aug 2021</i>

### Assay Name/Specification (minimum release criteria)

**Ligation and Recutting (Terminal Integrity)** - After a 10-fold over-digestion of pBC4 DNA with TspMI, >95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 25°C. Of these ligated fragments, ≥75% can be recut with TspMI.

**Endonuclease Activity (Nicking)** - A 50 µl reaction in CutSmart® Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 5 units of TspMI incubated for 4 hours at 75°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

**Exonuclease Activity (Radioactivity Release)** - A 50 µl reaction in CutSmart® Buffer containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] *E. coli* DNA and a minimum of 50 units of TspMI incubated for 4 hours at 75°C releases <0.1% of the total radioactivity.

**Non-Specific DNase Activity (16 Hour)** - A 50 µl reaction in CutSmart® Buffer containing 1 µg of pBC4 DNA and a minimum of 5 units of TspMI incubated for 16 hours at 75°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

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