

## New England Biolabs Product Specification

<i>Product Name:</i>	<i>Vaccinia Capping System</i>
<i>Catalog #:</i>	<i>M2080S</i>
<i>Concentration:</i>	<i>10,000 units/ml</i>
<i>Unit Definition:</i>	<i>One unit of Vaccinia Capping Enzyme is defined as the amount of enzyme required to incorporate 10 pmol of (<math>\alpha^{32}P</math>) GTP into an 80 nt transcript in 1 hour at 37°C.</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20 °C</i>
<i>Storage Conditions:</i>	<i>100 mM NaCl , 20 mM Tris-HCl (pH 8.0), 1 mM DTT , 0.1 mM EDTA , 50 % Glycerol , 0.1 % Triton®X-100</i>
<i>Specification Version:</i>	<i>PS-M2080S v1.0</i>
<i>Effective Date:</i>	<i>07 Nov 2013</i>

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

**Endonuclease Activity (Nicking)** - A 50  $\mu$ l reaction in Capping Buffer containing 1  $\mu$ g of supercoiled PhiX174 DNA and a minimum of 10 units of Vaccinia Capping System incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

**Exonuclease Activity (Radioactivity Release)** - A 50  $\mu$ l reaction in Capping Buffer containing 1  $\mu$ g of a mixture of single and double-stranded [ $^3H$ ] *E. coli* DNA and a minimum of 10 units of Vaccinia Capping System incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

**Protein Purity Assay (SDS-PAGE)** - Vaccinia Capping System is  $\geq$  95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

**RNase Activity (Extended Digestion)** - A 10  $\mu$ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 10 units of Vaccinia Capping System is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.



Date 07 Nov 2013

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