### New England Biolabs Product Specification

<table>
<thead>
<tr>
<th>Product Name:</th>
<th>Vent® DNA Polymerase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalog #:</td>
<td>M0254S/L</td>
</tr>
<tr>
<td>Concentration:</td>
<td>2,000 units/ml</td>
</tr>
<tr>
<td>Unit Definition:</td>
<td>One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at 75°C.</td>
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<tr>
<td>Shelf Life:</td>
<td>24 months</td>
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<tr>
<td>Storage Temp:</td>
<td>-20°C</td>
</tr>
<tr>
<td>Storage Conditions:</td>
<td>10 mM Tris-Cl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.1% Triton®X-100, 50% Glycerol, (pH 7.4 @ 25°C)</td>
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<tr>
<td>Specification Version</td>
<td>PS-M0254S/L v1.0</td>
</tr>
<tr>
<td>Effective Date:</td>
<td>24 Nov 2015</td>
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</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Endonuclease Activity (Nicking, Polymerase, dNTP) - A 50 µl reaction in ThermoPol® Reaction Buffer in the presence of 400 µM dNTPs containing 1 µg of supercoiled pUC19 DNA and a minimum of 20 units of Vent® DNA Polymerase incubated for 4 hours at 37°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</th>
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<tbody>
<tr>
<td>PCR Amplification (2.0 kb Lambda DNA) - A 25 µl reaction in ThermoPol® Reaction Buffer in the presence of 200 µM dNTPs and 0.5 µM primers containing 5 ng Lambda DNA with 0.25 units of Vent® DNA Polymerase for 25 cycles of PCR amplification results in the expected 2.0 kb product.</td>
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<td>Phosphatase Activity (pNPP) - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl₂ containing 2.5 mM p-Nitrophenyl Phosphate (pNPP) and a minimum of 100 units Vent® DNA Polymerase incubated for 4 hours at 37°C yields &lt;0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.</td>
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<tr>
<td>Protein Purity Assay (SDS-PAGE) - Vent® DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</td>
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<tr>
<td>qPCR DNA Contamination (E. coli Genomic) - A minimum of 2 units of Vent® DNA Polymerase is screened for the presence of E. coli genomic DNA using SYBR® Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is ≤ 1 E. coli genome.</td>
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<tr>
<td>RNase Activity (Extended Digestion) - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 µl of Vent® DNA Polymerase is incubated at 37°C. After incubation for 16 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</td>
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</tbody>
</table>
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Derek Robinson
Director of Quality Control

Date 24 Nov 2015