### Endonuclease Activity (Nicking)
- A 50 µl reaction in ThermoPol® Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 500 units of Bst 2.0 DNA Polymerase incubated for 4 hours at 65°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis. **Pass**

### Exonuclease Activity (Radioactivity Release)
- A 50 µl reaction in ThermoPol® Reaction Buffer containing 1 µg of a mixture of single and double-stranded [³H]E. coli DNA and a minimum of 500 units of Bst 2.0 DNA Polymerase incubated for 4 hours at 65°C releases <0.1% of the total radioactivity. **Pass**

### Non-Specific DNase Activity (16 Hour)
- A 50 µl reaction in NEBuffer 2 containing 1 µg of T3 DNA in addition to a reaction containing Lambda-HindIII DNA and a minimum of 120 units of Bst 2.0 DNA Polymerase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. **Pass**

### 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 Bst 2.0 DNA Polymerase incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis. **Pass**

### Protein Purity Assay (SDS-PAGE)
- Bst 2.0 DNA Polymerase is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. **Pass**
<table>
<thead>
<tr>
<th>Assay Name/Specification (minimum release criteria)</th>
<th>Lot #0071712</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>qPCR DNA Contamination (E. coli Genomic)</strong> - A minimum of 120 units of Bst 2.0 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>
<td>Pass</td>
</tr>
<tr>
<td><strong>RNase Activity (Extended Digestion)</strong> - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 µl of Bst 2.0 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>
<td>Pass</td>
</tr>
</tbody>
</table>

Authorized by
Melanie Fortier
18 May 2016

Inspected by
Tony Spear-Alfonso
30 Nov 2017