**Bst 2.0 DNA Polymerase**

1,600 units 8,000 U/ml Lot: 0071212

Recombinant Store at −20°C Exp: 12/14

**Description:** Bst 2.0 DNA Polymerase is an in silico designed homologue of *Bacillus stearothermophilus* DNA Polymerase I, Large Fragment (Bst DNA Polymerase, Large Fragment). Bst 2.0 DNA Polymerase contains 5′→3′ DNA polymerase activity and strong strand-displacement activity but lacks 5′→3′ exonuclease activity. Bst 2.0 DNA Polymerase displays improved amplification speed, yield, salt tolerance and thermostability compared to wild-type Bst DNA Polymerase, Large Fragment.

**Source:** Bst 2.0 DNA Polymerase is prepared from an *E. coli* strain that expresses the Bst 2.0 DNA Polymerase protein from an inducible promoter.

**Applications:**
- Isothermal DNA amplification
- Applications requiring strand-displacement DNA synthesis
- DNA sequencing through high GC regions
- Rapid sequencing from nanogram amounts of DNA template

**Reagents Supplied with Enzyme:**
- Isothermal Amplification Buffer (10X)

**Reaction Conditions:**
Specific reaction conditions will vary for different isothermal amplification applications. For best results, use 1X Isothermal Amplification Buffer.

**Incubate at 65°C.**

1X Isothermal Amplification Buffer:
- 20 mM Tris-HCl
- 50 mM KCl
- 2 mM MgSO₄
- 0.1% Tween-20
- pH 8.8 @ 25°C

**Unit Definition:**
One unit is defined as the amount of enzyme that will incorporate 25 nmol of dNTP into acid insoluble material in 30 minutes at 65°C.

**Unit Assay Conditions:**
- 50 mM KCl, 10 mM Tris-HCl (pH 8.8), 10 mM MgCl₂, 30 mM MgCl₂, 100 µM each dNTP, 100 µM dATP, 100 µM dGTP, 100 µM dUTP including [³H]-dUTP and 100 µg/ml BSA.

**Heat Inactivation:**
80°C for 20 minutes.

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**Quality Control Assays**

**Exonuclease Assay:** Incubation of a 50 µl reaction in 1X ThermoPol™ Reaction Buffer containing a minimum of 500 units of Bst 2.0 DNA Polymerase with 1 µg of a mixture of single and double-stranded [³H]-*E. coli* DNA (10⁶ cpms/µg) for 4 hours at 65°C releases < 0.1% of the total radioactivity.

**Endonuclease Assay:** Incubation of a 50 µl reaction in 1X ThermoPol Reaction Buffer containing a minimum of 500 units of Bst 2.0 DNA Polymerase with 1 µg of supercoiled φX174 DNA for 4 hours at 65°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

**Physical Purity:** Purified to > 99% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

(see other side)
**Phosphatase Assay:** Incubation of a 200 µl reaction in 1 M Diethanolamine (pH 9.8) and 0.5 mM MgCl₂ containing 2.5 mM p-Nitrophenol Phosphate and a minimum of 100 units of Bst 2.0 DNA Polymerase incubated for 4 hours at 37°C yields no detectable phosphatase activity as determined by spectrophotometric analysis of released p-nitrophenylene anion at 405 nm.

**RNase Activity:** Incubation of a 10 µl reaction in 1X NEBuffer 4 containing a minimum of 1 µl of Bst 2.0 DNA Polymerase and 40 ng of F-300 RNA transcript incubated for 16 hours at 37°C results in < 10% substrate degradation as determined by gel electrophoresis using fluorescent detection.

**Enzyme Properties**

<table>
<thead>
<tr>
<th>Activity in NEBuffers</th>
<th>Activity in NEBuffers</th>
</tr>
</thead>
<tbody>
<tr>
<td>ThermoPol Buffer</td>
<td>125%</td>
</tr>
<tr>
<td>Unit Assay Conditions</td>
<td>100%</td>
</tr>
<tr>
<td>NEBuffer 1</td>
<td>25%</td>
</tr>
<tr>
<td>NEBuffer 2</td>
<td>100%</td>
</tr>
<tr>
<td>NEBuffer 3</td>
<td>100%</td>
</tr>
<tr>
<td>NEBuffer 4</td>
<td>100%</td>
</tr>
<tr>
<td>NEBuffer EcoRI</td>
<td>100%</td>
</tr>
</tbody>
</table>

**Notes On Use:** Bst 2.0 DNA Polymerase does not exhibit 3´→5´ exonuclease activity.

Reaction temperatures above 70°C are not recommended.

Bst 2.0 DNA Polymerase cannot be used for thermal cycle sequencing or PCR.

**Companion Products Sold Separately:**

- **Bst 2.0 WarmStart™ DNA Polymerase**
  - #M0538S 1,600 units
  - #M0538L 8,000 units
  - #M0538M 8,000 units

- **Bst DNA Polymerase, Large Fragment**
  - #M0275S 1,600 units
  - #M0275L 8,000 units
  - #M0275M 8,000 units

- **Magnesium Sulfate (MgSO₄) Solution**
  - #B1003S 6.0 ml

- **Isothermal Amplification Buffer Pack**
  - #B0537S 6.0 ml

- **Deoxynucleotide Solution Set**
  - #N0446S 25 µmol each
  - #N0447S 8 µmol each
  - #N0447L 40 µmol each

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