SilverStar DNA polymerase
ME-0074-05 • ME-0074-SA

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Source and Description

SilverStar DNA Polymerase is a high-performance thermostable DNA polymerase purified from *Thermus aquaticus* (1). SilverStar offers consistent results across a wide range of assays. SilverStar leaves an A’ overhang such that the primer extension product is suitable for effective integration into TA cloning vectors.

Quality control

Each lot is tested for the absence of nicking and priming activities, exonucleases and non-specific endonucleases.

Shipping conditions

Product is shipped at +4°C. However, due to high stability features, trial samples are shipped at room temperature and we recommend usage of trial sample within the following 3-4 weeks.

Storage conditions

SilverStar DNA Polymerase can be stored at -20°C, in a constant temperature freezer for 12 months (1). SilverStar will remain stable if stored as specified. Repeated freeze-thaw cycles will affect the stability of Reaction Buffer. The Buffer will remain stable at +4°C for a minimum of one month.

Package contents

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
<th>Units</th>
<th>Concentration</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SilverStar DNA polymerase</td>
<td>100 µl</td>
<td>500 U</td>
<td>5 U/µl</td>
<td>DNA polymerase ME-0074-05</td>
</tr>
<tr>
<td></td>
<td>5 µl</td>
<td>25 U</td>
<td>5 U/µl</td>
<td>DNA polymerase ME-0074-SA</td>
</tr>
<tr>
<td>10x reaction buffer (yellow cap)</td>
<td>2x 1.5 ml (ME-0074-05)</td>
<td>10 x</td>
<td>160 mM (NH₄)₂SO₄, 670 mM Tris-HCl (pH 8.8 at 25°C), 0.1% Tween-20. Without MgCl₂</td>
<td></td>
</tr>
<tr>
<td>MgCl₂</td>
<td>2x 1.5 ml (ME-0074-05)</td>
<td>10 x</td>
<td>50 mM</td>
<td>MgCl₂ stock solution</td>
</tr>
<tr>
<td></td>
<td>1x 1.5 ml (ME-0074-SA)</td>
<td>10 x</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis conditions

25 mM TAPS, pH 9.3 (at 25°C); 50 mM KCl; 2 mM MgCl₂; 1 mM β-mercaptoethanol; 250 µM each dCTP, dGTP, dTTP; 250 µM (³H) dATP (0.05 Ci/µmol); activated salmon sperm DNA (1.25 µg/µl); total volume of 50 µl.

Associated activities

Endonuclease and exonuclease activities were not detectable after 2 and 1-hour incubations, respectively, of 1 µg lambda DNA and 0.22 µg of EcoRI-digested lambda DNA at 72°C in the presence of 15-20 units of SilverStar DNA polymerase.

Unit definition

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid-insoluble form in 30 minutes at 72°C.

Reaction Conditions

For a 50 µl volume

- 10x Reaction Buffer 5 µl
- 50mM MgCl₂ Solution 1.5 – 4.0 µl
- dNTP final concentration 250 – 500 µM each dNTP
- >20mM dNTP Mix (related product) 2.5 – 5 µl
- Template and Primers as required
- Enzyme 0.5 – 1.0 µl
- Water up to 50 µl
Always keeping on ice
MgCl₂ concentration: this polymerase is a magnesium-dependent enzyme. The supplied 50 mM MgCl₂ solution should be used to adjust magnesium ion concentration. Excessive amount of Mg²⁺ stabilizes the DNA double strand and consequently prevents complete denaturation of DNA, which reduces the extension yield. It may also stabilize spurious primer/template annealing, thus decreasing specificity.

Cycling conditions
Denaturation 94-96°C
Annealing see below
Elongation suggested 15-30 sec/kb at 70-72°C

Time and temperature for denaturation and annealing steps depend on the type of machine and primers. We advise that you check primer design using primer design software.

This data is intended for use as a guide only; conditions will vary from reaction to reaction and may need optimisation.

Troubleshooting guide

<table>
<thead>
<tr>
<th>Observation</th>
<th>Recommended solution(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No or low yield of extended product</td>
<td>Enzyme concentration too low - increase the amount enzyme in 0.5 U increments. Magnesium concentration too low – increase concentration in 0.25 mM increments. Primer annealing too low – Increase annealing temperature</td>
</tr>
<tr>
<td>Appearance of a smear</td>
<td>Reduce the quantity of polymerase. This should not exceed 1 U/50 µl, except for long fragments or not highly purified DNA</td>
</tr>
<tr>
<td>Appearance of contaminating bands</td>
<td>Reduce the magnesium concentration to 1.5 mM, or use Hot start technique</td>
</tr>
</tbody>
</table>

For further information please contact our Customer Help Desk:

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