StellarTaq™ DNA Polymerase

Product Description

StellarTaq DNA Polymerase has been engineered for speed, extreme inhibitor tolerance and specificity. The polymerase catalyzes 5'→3' DNA synthesis, has 5'→3' exonuclease activity and is deficient in 3'→5' exonuclease activity making it suitable for probe digestion. StellarTaq DNA Polymerase amplifies uracil-containing templates, incorporates modified bases, and performs A-tailing on DNA products.

StellarTaq DNA Polymerase is available in hot start (StellarTaq Hot Start DNA Polymerase) and non-hot start (StellarTaq DNA Polymerase) formats. For applications requiring specificity, we recommend StellarTaq Hot Start DNA Polymerase. The hot start mechanism inhibits 5'→3' polymerase activity and 5'→3' exonuclease activity, ensuring high target specificity even when amplification reactions are set up at room temperature.

StellarTaq DNA Polymerase, both hot start and non-hot start are also available in glycerol-free format.

Table of Contents

Product Description 1
Kit Contents 1
Relevant Taq DNA Polymerase Applications 2
Unit Definition and Buffer Composition 2
Storage and Handling 2
Recommended Reaction Setup 2
Recommended Cycling Protocol 2
Initial Reaction Optimization 3
References 3
Revision History 3

Kit Contents

Kit code	Description	Component Volumes (µL)	kU amount
Hot Start formats			
7K0117-50UL	StellarTaq Hot Start DNA Polymerase (5 U/µL)	50	0.25
7K0117-200UL	StellarTaq Hot Start DNA Polymerase (5 U/µL)	200	1
7K0121-50UL	StellarTaq Hot Start DNA Polymerase – Glycerol-Free (30 U/µL)	50	1.5
7K0120-50UL	StellarTaq Hot Start DNA Polymerase – Glycerol-Free (140 U/µL)	50	7
Non-Hot Start formats			
7K0116-50UL	StellarTaq DNA Polymerase (5 U/µL)	50	0.25
7K0116-200UL	StellarTaq DNA Polymerase (5 U/µL)	200	1
7K0118-50UL	StellarTaq DNA Polymerase – Glycerol-Free (140 U/µL)	50	7

For custom formats, contact the **Sales Team** at sales@watchmakergenomics.com.

Relevant Taq DNA Polymerase Applications

- Pathogen detection, including infectious diseases[1,2]
- PCR amplification of DNA fragments (≤5 kb)
- Probe and intercalating dye-based qPCR[3]
- RT-qPCR[4]
- PCR applications where inhibitors are present[5]
- Fast PCR[6]
- PCR applications where specificity is important[7]

Unit Definition and Buffer Composition

- 1 unit of StellarTaq DNA Polymerase incorporates 16 nmol of dNTPs into a DNA template in 30 minutes at 72°C.
- Storage buffer:
  - Glycerol-containing: 50 mM Tris-HCl, pH 7.5, 100 mM KCl, 0.1 mM EDTA, 50% Glycerol, 0.05% Tween 20
  - Glycerol-free: 50 mM Tris-HCl, pH 7.5, 300 mM KCl, 0.05% Tween 20
- Recommended StellarTaq DNA Polymerase 10X Reaction Buffer (not provided with kit): 200 mM Tris-HCl, pH 8.3, 1000 mM KCl, 0.04% Tween 20

Storage and Handling

- Glycerol-containing kits (K0116 and K0117) are shipped on ice packs. Upon receipt, store all kit components at -25°C to -15°C.
- Glycerol-free kits (K0118, K0120 and K0121) are shipped on dry ice. Upon receipt, store all standard kit components at -70°C to -90°C.

Keep all components and reaction mixes on ice or a cooled reagent block during routine use. Take care to mix solutions thoroughly before use and during reaction setup. Do not vortex the polymerase. When stored and handled as indicated, the product will retain full performance until the expiry date printed on the kit box.

Recommended Reaction Setup

Component	Final Concentration	Volume (25 µL reaction)
Buffer (10X)[1]	1X	2.5 µL
$MgCl_2$ (25 mM)[2]	2 mM (1.5 – 6 mM)	2 µL
Each primer (10 µM Fwd/Rev)	0.2 µM	0.5 µL
StellarTaq DNA Polymerase (5 U/µL)[3]	0.012 U/µL (0.006 – 0.12 U/µL)	0.06 µL
dNTPs (10 mM each)	0.2 mM	0.5 µL
Template DNA[4]	Genomic 1 – 50 ng Plasmid and viral 1 pg – 1 ng	Variable
PCR grade water	–	Up to 25 µL

[1]Not supplied with the kit. The enzyme performs well in a range of buffers, but will require higher salt concentrations than wild-type *Taq* DNA Polymerase. A recommended 10X buffer is 200 mM Tris-HCl, pH 8.3, 1000 mM KCl, 0.04% Tween 20. See **Initial Reaction Optimization** below for optimization of salt and enzyme concentrations for specific applications.

[2]A final $MgCl_2$ concentration of 2 mM is suggested for endpoint PCR or intercalating dye based qPCR. Increased concentrations of $MgCl_2$ will be needed for probe-based qPCR and multiplex PCR. The $MgCl_2$ concentration may need to be optimized.

[3]We recommend using 0.012 U/µL as a final enzyme concentration. Enzyme concentration is an important parameter affecting performance and the optimal concentration will vary depending on the application. Use higher concentrations of enzyme—up to 0.12 U/µL—in reactions with inhibitors, reverse transcriptase, for fast PCR, or when no amplification is observed. Use lower concentrations of enzyme—down to 0.012 U/µL—if non-specific amplification is observed.

[4]Template DNA input is assay-dependent and must be optimized.

Recommended Cycling Protocol

Step	Temperature (°C)	Time (sec)	Cycles
Initial denaturation[1]	95	60	1
Denaturation	95	5	25 – 35[4]
Annealing and extension[2]	~60[3]	10s/2 kb	
–	12	Hold	–

[1]Initial denaturation time depends on sample type. A 60-second denaturation time should be used as a first approach. Complex samples may require extended denaturation time (3 – 5 minutes).

[2]A 10-second combined annealing and extension time is appropriate for targets ≤2 kb. Applications requiring fast PCR can be optimized by decreasing the combined annealing and extension time and/or by increasing the enzyme concentration. Traditional three-step PCR can also be performed.

[3]The annealing temperature is dependent on the primer(s) sequence(s). Optimal annealing temperature is determined empirically in an annealing temperature gradient experiment with a gradient range from 55°C to 72°C.

[4]Cycle number varies based on DNA input.

Initial Reaction Optimization

StellarTaq DNA Polymerase is an engineered polymerase with increased activity and requires higher salt concentration for optimal performance. It will not perform optimally in a wild-type *Taq* DNA Polymerase buffer as the salt concentration is too low. We recommend conducting an initial salt concentration vs. enzyme concentration matrix experiment to identify the optimal salt and enzyme concentration for your assay. Figure 1 illustrates such a matrix and the expected result.

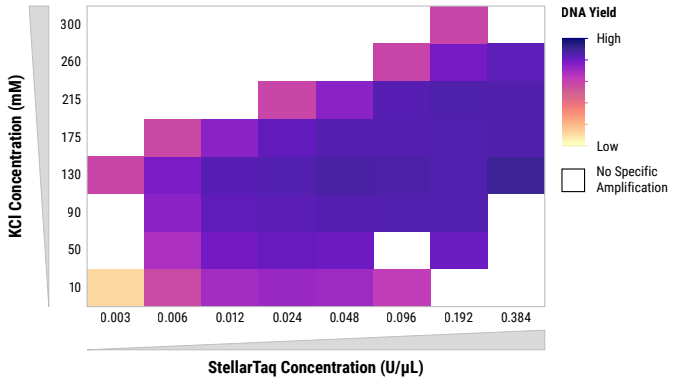

Figure 1: A potassium chloride (KCl) vs StellarTaq DNA Polymerase matrix experiment. A typical scouting experiment can range from an 8x8 matrix as is shown here down to a 4x4 matrix (e.g., 70, 105, 140 and 175 mM salt and 0.012, 0.04, 0.07, and 0.1 U/µL enzyme). Larger matrices enable finer resolution for identifying conditions with the highest DNA yield. This matrix explores the boundaries of the reaction space. At high enzyme and low salt concentrations, non-specific amplification occurs with all polymerases (a DNA smear will appear on agarose gel electrophoresis). At low enzyme and high salt concentrations, no product will be visualized as high salt concentration prevents amplification. The assay conditions with the highest DNA yield will be found along a diagonal range from low salt and low enzyme concentrations to high salt and high enzyme concentrations.

References

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Revision History

Version	Description	Date
1.0	First protocol release	12/2024

WATCHMAKER GENOMICS

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