Q5® Hot Start High-Fidelity DNA Polymerase

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M0493S 006141216121

M0493S

100 units **2,000 U/ml** **Lot: 0061412**
RECOMBINANT **Store at –20°C** **Exp: 12/16**

Description: Q5 Hot Start High-Fidelity DNA Polymerase is a high-fidelity, thermostable, hot start DNA polymerase with 3´→ 5´ exonuclease activity, fused to a processivity-enhancing Sso7d domain to support robust DNA amplification. The addition of an aptamer-based inhibitor allows room temperature reaction setup. With an error rate > 100-fold lower than that of *Taq* DNA Polymerase and 12-fold lower than that of *Pyrococcus furiosus* (*Pfu*) DNA Polymerase, Q5 Hot Start High-Fidelity DNA Polymerase is ideal for cloning and can be used for long or difficult amplicons. Q5 Hot Start High-Fidelity DNA Polymerase is supplied with an optimized buffer system that allows robust amplification regardless of GC content. The 5X Q5 Reaction Buffer contains 2 mM $MgCl_2$ at final (1X) reaction concentrations and is recommended for most routine applications. For GC-rich targets (≥ 65% GC), amplification can be improved by the addition of the 5X Q5 High GC Enhancer. Q5 Hot Start High-Fidelity DNA Polymerase is unlike typical, lower fidelity PCR enzymes. To determine the optimal annealing temperatures for a given set of primers, use of the **NEB T_m Calculator** is highly recommended (www.neb.com/Tmcalculator).

Source: An *E. coli* strain that carries the Q5 High-Fidelity DNA Polymerase gene.

Applications:

- High-specificity PCR
- High-fidelity PCR
- Cloning
- Long or difficult amplification
- High-throughput PCR

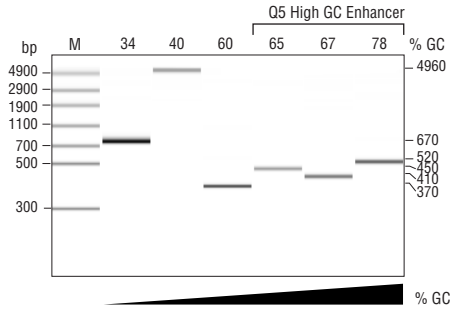
Reagents Supplied with Enzyme:
5X Q5 Reaction Buffer
5X Q5 High GC Enhancer

Reaction Conditions: 1X Q5 Reaction Buffer, DNA template, 0.5 µM primers, 200 µM dNTPs (not included), 1X Q5 High GC Enhancer (optional) and 1 unit of Q5 Hot Start High-Fidelity DNA Polymerase in a total reaction volume of 50 µl.

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 74°C.

Unit Assay Conditions: 25 mM TAPS-HCl (pH 9.3 @ 25°C), 50 mM KCl, 2 mM $MgCl_2$, 1 mM β-mercaptoethanol, 200 µM dNTPs including [^{3}H]-dTTP and 400 µg/ml activated Calf Thymus DNA.

Heat Inactivation: No



Amplification of a variety of human genomic amplicons from low to high GC content using Q5 Hot Start High-Fidelity DNA Polymerase. All reactions were set up at room temperature, conducted using 30 cycles of amplification and visualized by microfluidic LabChip® analysis.

Quality Control Assays

7 kb Genomic DNA PCR: 30 cycles of PCR amplification in a 50 µl reaction containing 20 ng genomic DNA with 1.0 unit of Q5 Hot Start High-Fidelity DNA Polymerase in the presence of 200 µM dNTPs and 0.5 µM of each primer in Q5 Reaction Buffer result in the expected 7 kb product.

20 kb Lambda DNA PCR: 22 cycles of PCR amplification in a 50 µl reaction containing 10 ng Lambda DNA with 1.0 unit of Q5 Hot Start High-Fidelity DNA Polymerase in the presence of 200 µM dNTPs and 1.0 µM of each primer in Q5 Reaction Buffer result in the expected 20 kb product.

Enhancer-Dependent High GC (65% GC) PCR: 30 cycles of PCR amplification in a 50 µl reaction containing 20 ng genomic DNA with 1.0 unit of Q5 Hot Start High-Fidelity DNA Polymerase in the presence of 200 µM dNTPs, 1X Q5 High GC Enhancer and 0.5 µM of each primer in Q5 Reaction Buffer result in the enhancer-dependent production of the 452 bp high GC product.

Hot Start-Specific Genomic DNA PCR: 25 cycles of PCR amplification in a 25 µl reaction containing 50 ng genomic DNA with 0.5 units of Q5 Hot Start High-Fidelity DNA Polymerase in the presence of 200 µM dNTPs, 1X Q5 High GC Enhancer and 0.5 µM of each primer in Q5 Reaction Buffer result in the expected 665 bp product, free of non-specific amplification products after pre-incubation at room temperature for 1 hour.

Endonuclease Activity: Incubation of a 50 µl reaction in NEBuffer 2 containing a minimum of 10 units of Q5 High-Fidelity DNA Polymerase with 200 µM dNTPs and 1 µg of supercoiled pUC19 DNA for 4 hours at either 37°C or 72°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

Physical Purity: Purified to > 97% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

qPCR DNA Contamination (*E.coli* Genomic): A minimum of 4 units of Q5 High-Fidelity 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 less than 1 *E. coli* genome.

PCR

Please note that protocols with Q5 Hot Start High-Fidelity DNA Polymerase may differ from protocols with other polymerases. Conditions recommended below should be used for optimal performance.

Reaction Setup:
Q5 Hot Start High-Fidelity DNA Polymerase is inhibited at room temperature, allowing flexible reaction setup (RT or ice).

All components should be mixed prior to use. Q5 Hot Start High-Fidelity DNA Polymerase may be diluted in 1X Q5 Reaction Buffer just prior to use in order to reduce pipetting errors.

COMPONENT	25 µl REACTION	50 µl REACTION	FINAL CONCENTRATION
5X Q5 Reaction Buffer	5 µl	10 µl	1X
10 mM dNTPs	0.5 µl	1 µl	200 µM
10 µM Forward Primer	1.25 µl	2.5 µl	0.5 µM
10 µM Reverse Primer	1.25 µl	2.5 µl	0.5 µM
Template DNA	variable	variable	<1,000 ng
Q5 Hot Start High-Fidelity DNA Polymerase	0.25 µl	0.5 µl	0.02 U/µl
5X Q5 High GC Enhancer (optional)	(5 µl)	(10 µl)	(1X)
Nuclease-Free Water	to 25 µl	to 50 µl	

Notes: Gently mix the reaction. Collect all liquid to the bottom of the tube by a quick spin if necessary. Overlay the sample with mineral oil if using a PCR machine without a heated lid.

Transfer PCR tubes to a PCR machine and begin thermocycling.

Q5 Hot Start High-Fidelity DNA Polymerase does not require a separate activation step. Standard Q5 cycling conditions are recommended.

Thermocycling Conditions for a Routine PCR:

STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
25–35 Cycles	98°C *50–72°C 72°C	5–10 seconds 10–30 seconds 20–30 seconds/kb
Final Extension	72°C	2 minutes
Hold	4–10°C	

*Use of the NEB T_m Calculator is highly recommended.

General Guidelines:

1. Template:
   Use of high quality, purified DNA templates greatly enhances the success of PCR. Recommended amounts of DNA template for a 50 µl reaction are as follows:

DNA	AMOUNT
Genomic	1 ng–1 µg
Plasmid or Viral	1 pg–1 ng

2. Primers:
   Oligonucleotide primers are generally 20–40 nucleotides in length and ideally have a GC content of 40–60%. Computer programs such as Primer3 (http://frodo.wi.mit.edu/primer3) can be used to design or analyze primers. The best results are typically seen when using each primer at a final concentration of 0.5 µM in the reaction.

(see other side)

CERTIFICATE OF ANALYSIS

3. Mg^{++} and additives:
   Mg^{++} concentration of 2.0 mM is optimal for most PCR products generated with Q5 Hot Start High-Fidelity DNA Polymerase. When used at a final concentration of 1X, the Q5 Reaction Buffer provides the optimal Mg^{++} concentration.

   Amplification of some difficult targets, like GC-rich sequences, may be improved by the addition of 1X Q5 High GC Enhancer. The Q5 High GC Enhancer is not a buffer and should not be used alone. It should be added only to reactions with the Q5 Reaction Buffer when other conditions have failed.

4. Deoxynucleotides:
   The final concentration of dNTPs is typically 200 µM of each deoxynucleotide. Q5 Hot Start High-Fidelity DNA Polymerase cannot incorporate dUTP and is not recommended for use with uracil-containing primers or templates.

5. Q5 Hot Start High-Fidelity DNA Polymerase concentration:
   We generally recommend using Q5 Hot Start High-Fidelity DNA Polymerase at a final concentration of 20 units/ml (1.0 unit/50 µl reaction). However, the optimal concentration of Q5 Hot Start High-Fidelity DNA Polymerase may vary from 10–40 units/ml (0.5–2 units/50 µl reaction) depending on amplicon length and difficulty. Do not exceed 2 units/50 µl reaction.

6. Buffers:
   The 5X Q5 Reaction Buffer provided with the enzyme is recommended as the first-choice buffer for robust, high-fidelity amplification. For difficult amplicons, such as GC-rich templates or those with secondary structure, the addition of the Q5 High GC Enhancer can improve reaction performance. The 5X Q5 Reaction Buffer contains 2.0 mM $MgCl_2$ at the final (1X) concentration.

7. Denaturation:
   Q5 Hot Start High-Fidelity DNA Polymerase does not require a separate activation step.

   An initial denaturation of 30 seconds at 98°C is sufficient for most amplicons from pure DNA templates. Longer denaturation times can be used (up to 3 minutes) for templates that require it.

   During thermocycling, the denaturation step should be kept to a minimum. Typically, a 5–10 second denaturation at 98°C is recommended for most templates.

8. Annealing:
   Optimal annealing temperatures for Q5 Hot Start High-Fidelity DNA Polymerase tend to be higher than for other PCR polymerases. The **NEB T_m Calculator** should be used to determine the annealing temperature when using this enzyme. Typically, use a 10–30 seconds annealing step at 3°C above the T_m of the lower T_m primer. A temperature gradient can also be used to optimize the annealing temperature for each primer pair.

   For high T_m primer pairs, two-step cycling without a separate annealing step can be used (see note 11).

9. Extension:
   The recommended extension temperature is 72°C. Extension times are generally 20–30 seconds per kb for complex, genomic samples, but can be reduced to 10 seconds per kb for simple templates (plasmid, *E. coli*, etc.) or complex templates < 1 kb. Extension time can be increased to 40 seconds per kb for cDNA or long, complex templates, if necessary.

   A final extension of 2 minutes at 72°C is recommended.

10. Cycle number:
    Generally, 25–35 cycles yield sufficient product. For genomic amplicons, 30–35 cycles are recommended.

11. 2-step PCR:
    When primers with annealing temperatures ≥ 72°C are used, a 2-step thermocycling protocol (combining annealing and extension into one step) is possible.

12. Amplification of long products:
    When amplifying products > 6 kb, it is often helpful to increase the extension time to 40–50 seconds/kb.

13. PCR product:
    The PCR products generated using Q5 Hot Start High-Fidelity DNA Polymerase have blunt ends. If cloning is the next step, then blunt-end cloning is recommended. If T/A-cloning is preferred, the DNA should be purified prior to A-addition, as Q5 Hot Start High-Fidelity DNA Polymerase will degrade any overhangs generated.

    Addition of an untemplated -dA can be done with *Taq* DNA Polymerase (NEB #M0267) or Klenow exo⁻ (NEB #M0212).

Companion Products Sold Separately:

Q5 High-Fidelity DNA Polymerase

#M0491S	100 units
#M0491L	500 units

Q5 High-Fidelity 2X Master Mix

#M0492S	100 reactions
#M0492L	500 reactions

Q5 Hot Start High-Fidelity 2X Master Mix

#M0494S	100 reactions
#M0494L	500 reactions

Q5 Reaction Buffer Pack

#B9027S	6.0 ml

Deoxynucleotide Solution Set

#N0446S	25 µmol of each

Deoxynucleotide Solution Mix

#N0447S	8 µmol of each
#N0447L	40 µmol of each

Magnesium Chloride ($MgCl_2$) Solution

#B9021S	6.0 ml