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# PCR Using Q5® Hot Start High-Fidelity DNA Polymerase (M0493) V.2



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**We use this protocol and it's working**

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## Abstract

Q5<sup>®</sup> 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 ~280-fold lower than that of *Taq* 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 Mg<sup>++</sup> 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<sub>m</sub> Calculator** is highly recommended.

## Guidelines

### 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:

A	B
DNA	AMOUNT
DNA 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** 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.

#### 3. Mg<sup>++</sup> and additives:

Mg<sup>++</sup> concentration of 2.0 mM is optimal for most PCR products generated with Q5 High-Fidelity DNA Polymerase. When used at a final concentration of 1X, the Q5 Reaction Buffer provides the optimal Mg<sup>++</sup> 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, especially for amplicons longer than 5 kb.

#### 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 Mg<sup>++</sup> 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<sub>m</sub> 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<sub>m</sub> of the lower T<sub>m</sub> primer. A temperature gradient can also be used to optimize the annealing temperature for each primer pair.

For high T<sub>m</sub> 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**).


## Materials

### MATERIALS

 Q5 Hot Start High-Fidelity DNA Polymerase - 500 units **New England Biolabs Catalog #M0493L**

 Q5 Hot Start High-Fidelity DNA Polymerase - 100 units **New England Biolabs Catalog #M0493S**

## Safety warnings

 Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

## Before start

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.

1 Set up the following reaction:



**Note**

*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.

A	B	C	D
Component	25 $\mu$ l Reaction	50 $\mu$ l Reaction	Final Concentration
5X Q5 Reaction Buffer	5 $\mu$ l	10 $\mu$ l	1X
10 mM dNTPs	0.5 $\mu$ l	1 $\mu$ l	200 $\mu$ M
10 $\mu$ M Forward Primer	1.25 $\mu$ l	2.5 $\mu$ l	0.5 $\mu$ M
10 $\mu$ M Reverse Primer	1.25 $\mu$ l	2.5 $\mu$ l	0.5 $\mu$ M
Template DNA	variable	variable	< 1,000 ng
Q5 Hot Start High-Fidelity DNA Polymerase	0.25 $\mu$ l	0.5 $\mu$ l	0.02 U/ $\mu$ l
5X Q5 High GC Enhancer (optional)	(5 $\mu$ l)	(10 $\mu$ l)	(1X)
Nuclease-Free Water	to 25 $\mu$ l	to 50 $\mu$ l	

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



3 Transfer PCR tubes to a PCR machine and begin thermocycling:



**Note**

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:**

A	B	C
STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
25–35 Cycles	98°C	5–10 seconds

A	B	C
	*50–72°C	10–30 seconds
	72°C	20–30 seconds/kb
Final Extension	72°C	2 minutes
Hold	4–10°C	

\*Use of the **NEB T<sub>m</sub> Calculator** is highly recommended.