PCR Using Q5® Hot Start High-Fidelity DNA Polymerase (M0493)

New England Biolabs

Abstract

This protocols is for PCR using Q5® High-Fidelity DNA Polymerase (M0491)

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Guidelines

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
	98°C	5–10 seconds
25–35 Cycles	*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 Tm 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
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++ and additives:
Mg++ 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++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 MgCl2 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 Tm 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 Tm of the lower Tm primer. A temperature gradient can also be used to optimize the annealing temperature for each primer pair.

For high Tm 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

Q5 Hot Start High-Fidelity DNA Polymerase - 100 units M0493S by New England Biolabs

Protocol

Step 1.
Set up the following reaction:

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
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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	

PROTOCOL

. Mixture for M0493 Q5 PCR

CONTACT: New England Biolabs

NOTES

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Step 1.1.
5X Q5 Reaction Buffer

Step 1.2.
10 mM dNTPs

REAGENTS

Deoxynucleotide Solution Mix - 8 umol of each N0447S by New England Biolabs

Step 1.3.
10 µM Forward Primer

Step 1.4.
10 µM Reverse Primer

Step 1.5.

Template DNA

Step 1.6.
Q5 Hot Start High-Fidelity DNA Polymerase

Step 1.7.
Optional: 5X Q5 High GC Enhancer

Step 1.8.
Nuclease-Free Water

Step 2.
Gently mix the reaction.

Step 3.
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.

Step 4.
Transfer PCR tubes to a PCR machine and begin thermocycling.

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

Warnings

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