Protocol for Q5® Hot Start High-Fidelity 2X Master Mix

Please note that protocols with Q5 Hot Start High-Fidelity 2X Master Mix may differ from protocols with other polymerases. Conditions recommended below should be used for optimal performance.

Reaction Setup:
Q5 Hot Start High-Fidelity 2X Master Mix is inhibited at room temperature, allowing flexible reaction setup (RT or ice).

All components should be mixed prior to use.

Component	25 µl Reaction	50 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	12.5 µl	25 µl	1X
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
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.

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Q5 Hot Start High-Fidelity 2X Master Mix 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	5–10 seconds
	*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_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
DNA Genomic	1 ng–1 µg
Plasmid or Viral	1 pg–10 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:
   The Q5 High-Fidelity Master Mix contains 2.0 mM Mg^{++} when used at a 1X concentration. This is optimal for most PCR products generated with this master mix.

4. Deoxynucleotides:
   The final concentration of dNTPs is 200 µ M of each deoxynucleotide in the 1X Q5 Hot Start High-Fidelity Master Mix. 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:
   The concentration of Q5 Hot Start High- Fidelity DNA Polymerase in the Q5 Hot Start High-Fidelity 2X Master Mix has been optimized for best results under a wide range of conditions.

6. 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.

7. Annealing:
   Optimal annealing temperatures for Q5 Hot Start High-Fidelity 2X Master Mix 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 second 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).

8. 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.

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

10. 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.

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

12. PCR product:
    The PCR products generated using Q5 Hot Start High-Fidelity 2X Master Mix 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).

Links to this resource

Product Categories: Q5® High-Fidelity DNA Polymerases Products, Master Mixes Products

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