Q5® High-Fidelity 2X Master Mix

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M0492S 006140616061

M0492S

100 reactions (50 µl vol) **Lot: 0061406**

RECOMBINANT Store at –20°C Exp: 6/16

Description: The Q5 High-Fidelity 2X Master Mix offers robust, high-fidelity performance in a convenient master mix format. The Q5 High-Fidelity 2X Master Mix features a high-fidelity, thermostable DNA polymerase with 3´→ 5´ exonuclease activity, fused to a processivity-enhancing Sso7d domain to support robust DNA amplification. 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 High-Fidelity DNA Polymerase is ideal for cloning and can be used for long or difficult amplicons. The convenient master mix formulation is supplied at a 2X concentration. The mix contains dNTPs, Mg^{++} and a proprietary broad-use buffer requiring only the addition of primers and DNA template for robust amplification regardless of GC content. When used at the recommended 1X final concentration, the Q5 High-Fidelity Master Mix contains 2 mM $MgCl_2$. Q5 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).

Please Note: A precipitate (most noticeable after the first 1–2 freeze/thaw cycles) is not uncommon. To ensure optimal performance, the master mix should be thawed and resuspended prior to use. Stability testing using up to 20 freeze/thaw cycles has shown no negative effect on master mix performance.

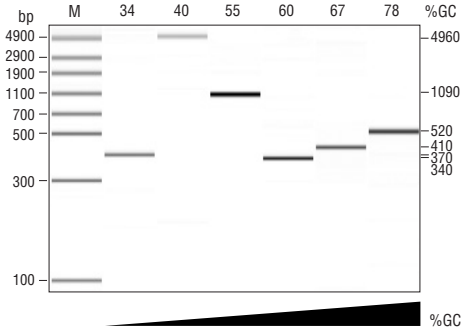
Source: An *E. coli* strain that carries the Q5 High-Fidelity DNA Polymerase gene.

Applications:

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

Reaction Conditions: 1X Q5 High-Fidelity Master Mix, DNA template and 0.5 µM primers in a total reaction volume of 50 µl.

Heat Inactivation: No



Amplification of a variety of human genomic amplicons from low to high GC content using Q5 High-Fidelity 2X Master Mix. All reactions were 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 1X Q5 High-Fidelity Master Mix and 0.5 µM of each primer 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 1X Q5 High-Fidelity Master Mix and 1.0 µM of each primer result in the expected 20 kb product.

Note: Product specifications for individual components in the Q5 High-Fidelity 2X Master Mix are available separately.

PCR

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

Reaction Setup:
We recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (98°C). 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.

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.

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 µM of each deoxynucleotide in the 1X Q5 High-Fidelity Master Mix. Q5 High-Fidelity DNA Polymerase cannot incorporate dUTP and is not recommended for use with uracil-containing primers or templates.

5. Q5 High-Fidelity DNA Polymerase concentration:
   The concentration of Q5 High-Fidelity DNA Polymerase in the Q5 High-Fidelity 2X Master Mix has been optimized for best results under a wide range of conditions.

6. Denaturation:
   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 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 10).

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

(see other side)

CERTIFICATE OF ANALYSIS

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 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 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 Hot Start High-Fidelity DNA Polymerase

#M0493S	100 units
#M0493L	500 units

Q5 High-Fidelity DNA Polymerase

#M0491S	100 units
#M0491L	500 units

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