INSTRUCTION MANUAL

Q5® High-Fidelity PCR Kit

NEB #E0555S/L

50/200 reactions

Version 4.0_1/20

Table of Contents

Kit Components

This kit contains a sufficient supply of master mix and nuclease-free water to perform 50 polymerase chain reactions (NEB #E0555S) or 200 reactions (NEB #E0555L). For added convenience, gel loading dye, and a Quick-Load Purple DNA Ladder are also included in this kit.

Q5 High-Fidelity 2X Master Mix (NEB #M0492) 1.25 ml/44 x 1.25 ml (2X)

Nuclease-free Water (NEB #B1502) 1.5 ml/4 x 1.5 ml (1X)

Nuclease-free Water is quality-tested for nuclease contamination and is supplied to ensure maximal amplification success.

Gel Loading Dye, Purple (NEB #B7024) 1 ml (6X)

Gel Loading Dye, Purple (6X) is a pre-mixed loading buffer which contains a combination of two dyes, Dye 1 (pink/red) and Dye 2 (blue). The red dye serves as the tracking dye for both agarose and non-denaturing polyacrylamide gel electrophoresis. The two dyes separate upon gel electrophoresis; the red band is the major indicator and migrates similarly to Bromophenol Blue on agarose gels. Specifically chosen, this dye does not leave a shadow under UV light. This solution contains SDS, and EDTA, which chelates magnesium (up to 10 mM) in enzymatic reactions, thereby stopping the reaction. The dye also contains Ficoll, which creates brighter and tighter bands when compared to glycerol loading dyes.

Quick-Load® Purple 1 kb Plus DNA Ladder (NEB #N0550) 0.2 ml/0.4 ml (100 µg/ml)

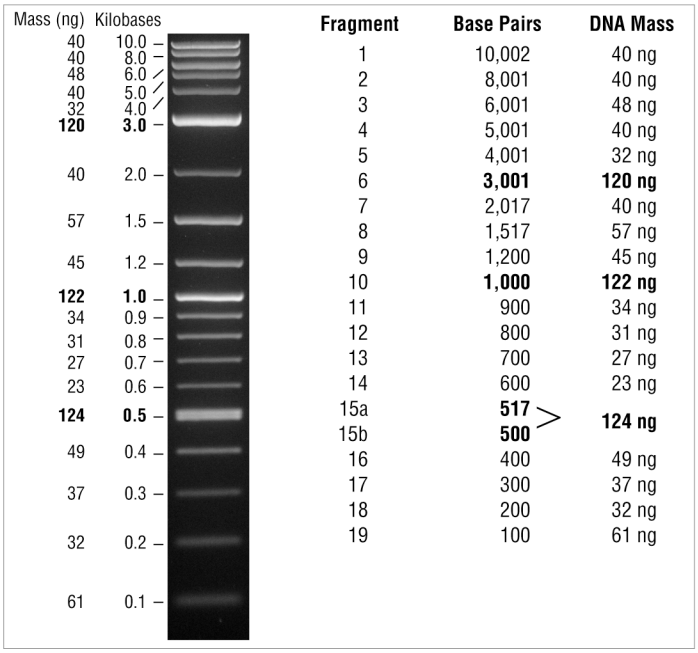
Quick-Load Purple 1 kb Plus DNA Ladder is a pre-mixed, ready-to-load molecular weight marker containing one tracking dye. Tracking dye does not leave a shadow under UV light.

The DNA Ladder consists of proprietary plasmids which are digested to completion with appropriate restriction enzymes to yield 19 bands suitable for use as molecular weight standards for agarose gel electrophoresis. This digested DNA includes fragments ranging from 100 bp to 10 kb. The 0.5, 1.0 and 3.0 kb bands have increased intensity to serve as reference bands.

Introduction

Q5 High-Fidelity DNA Polymerase is an ultra 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 Q5 High-Fidelity 2X Master Mix offers robust, high-fidelity performance in a convenient master mix format. 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 Tm Calculator is highly recommended** (www.neb.com/TmCalculator).



Fragment	Base Pairs	DNA Mass
1	10,002	40 ng
2	8,001	40 ng
3	6,001	48 ng
4	5,001	40 ng
5	4,001	32 ng
6	**3,001**	**120 ng**
7	2,017	40 ng
8	1,517	57 ng
9	1,200	45 ng
10	**1,000**	**122 ng**
11	900	34 ng
12	800	31 ng
13	700	27 ng
14	600	23 ng
15a	**517**	**124 ng**
15b	**500**	
16	400	49 ng
17	300	37 ng
18	200	32 ng
19	100	61 ng

Quick-Load Purple 1 kb Plus DNA Ladder visualized by ethidium bromide staining on a 1.0% TBE agarose gel. Mass values are for 1 µg/lane.

Protocol for Routine 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

1. 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.

	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 at the bottom of the tube with a quick spin, if necessary.

2. Transfer PCR tubes to a thermocycler and begin the cycling program.

3. 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 Tm Calculator is highly recommended (www.neb.com/tmcalculator).

Protocol for PCR Optimization:

The following guidelines are provided to ensure successful PCR using Q5 High-Fidelity DNA Polymerase. These guidelines cover routine PCR. Amplification of templates with high GC content, high secondary structure, low template concentrations or longer amplicons may require further optimization.

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-1µg
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/) 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 µM Mg^{++} when used at a 1X concentration. This is optimal for most PCR products generated with this 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 template.

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 DNA Polymerase tend to be higher than for other PCR polymerases. **The NEB Tm Calculator (www.neb.com/TmCalculator) should be used to determine the annealing temperature when this enzyme.** Typically, use a 10–30 second 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 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 time can be increased to 40 seconds per kb for cDNA templates, if necessary. A final extension of 2 minutes at 72°C is recommended.

9. Cycle number:
   Generally, 25–35 cycles yields 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.

Thermocycling conditions for a routine 2-step PCR:

STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
25-35 Cycles	98°C	5-10 seconds
	72°C	15-30 seconds/kb
Final Extension	72°C	2 minutes
Hold	4-10°C	∞

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 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 High-Fidelity DNA Polymerase will degrade any overhangs generated.
Addition of an untemplated -dA can be achieved with *Taq* DNA Polymerase (NEB #M0267) or Klenow Fragment (3′→5′ exo⁻) (NEB #M0212).

Troubleshooting Guide:

PROBLEM	POSSIBLE CAUSE	SOLUTION(S)
Sequence Errors	Suboptimal reaction conditions	• Reduce number of cycles • Decrease extension time
	Template DNA has been damaged	• Start with a fresh template • Try repairing DNA template with the PreCR® Repair Mix (NEB #M0309) • Limit UV exposure time when analyzing or excising PCR product from the gel
	Desired Sequence may be toxic to host	• Clone into a non-expression vector • Use a low copy number cloning vector
Incorrect Product Size	Incorrect annealing temperature	• Recalculate primer Tm values using the NEB Tm calculator (www.neb.com/TmCalculator)
	Mispriming	• Verify that primers have no additional complementary regions within the template DNA
	Nuclease contamination	• Repeat reactions using fresh solutions
No Product	Incorrect annealing temperature	• Recalculate primer Tm values using the NEB Tm calculator (www.neb.com/TmCalculator) • Test an annealing temperature gradient, starting at 5°C below the lower Tm of the primer pair
	Poor primer design	• Check specific product literature for recommended primer design • Verify that primers are non-complementary, both internally and to each other • Increase length of primer
	Poor primer specificity	• Verify that oligos are complementary to proper target sequence
	Insufficient primer concentration	• Verify that each primer is added to a final concentration of 0.5 µM
	Missing reaction component	• Repeat reaction setup
	Poor template quality	• Analyze DNA via gel electrophoresis before and after incubation with Mg^{++} • Check 260/280 ratio of DNA template
	Presence of inhibitor in reaction	• Further purify starting template by alcohol precipitation, drop dialysis or commercial clean-up kit • Decrease sample volume
	Insufficient number of cycles	• Rerun the reaction with more cycles

PROBLEM	POSSIBLE CAUSE	SOLUTION(S)
No Product	Incorrect thermocycler programming	• Check program, verify times and temperatures
	Inconsistent thermocycler block temperature	• Test calibration of heating block
	Contamination of reaction tubes or solutions	• Autoclave empty reaction tubes prior to use to eliminate biological inhibitors • Prepare fresh solutions or use new reagents
Multiple or non-specific products	Premature replication	• Use a hot start polymerase, such as Q5 Hot Start High-Fidelity DNA Polymerase (NEB #M0493) • Set up reactions on ice using chilled components and add samples to thermocycler preheated to the denaturation temperature
	Primer annealing temperature too low	• Increase annealing temperature
	Poor primer design	• Check specific product literature for recommended primer design • Verify that primers are non-complementary, both internally and to each other • Increase length of primer • Avoid GC-rich 3′ ends
	Incorrect primer concentration	• Verify that each primer is added to a final concentration of 0.5 µM
	Contamination with exogenous DNA	• Use positive-displacement pipettes or non-aerosol tips • Set up dedicated work area and pipettor for reaction setup • Wear gloves during reaction setup
	Incorrect template concentration	• For low complexity templates (i.e., plasmid, lambda, BAC DNA), use 1 pg–1 ng of DNA per 50 µl reaction • For higher complexity templates (i.e., genomic DNA), use 1 ng–1 µg of DNA per 50 µl reaction

Quality Controls

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 2X 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 2X 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 PCR Kit are available separately.

Ordering Information

NEB #	PRODUCT	SIZE
E0555S/L	Q5 High-Fidelity PCR Kit	50/200 reactions
KIT COMPONENTS SOLD SEPARATELY		
M0492S/L	Q5 High-Fidelity 2X Master Mix	100/500 reactions
B7024S	Gel Loading Dye, Purple (6X)	4 ml
N0550S	Quick-Load Purple 1 kb Plus DNA Ladder	1.25 ml

COMPANION PRODUCTS

NEB #	PRODUCT	SIZE
M0491S/L	Q5 High-Fidelity DNA Polymerase	100/500 units
M0493S/L	Q5 Hot Start High-Fidelity DNA Polymerase	100/500 units
M0494S/L	Q5 Hot Start High-Fidelity 2X Master Mix	100/500 units
N0446S	Deoxynucleotide (dNTP) Solution Set	25 µmol of each
N0447S/L	Deoxynucleotide (dNTP) Solution Mix	8/40 µmol of each
B9027S	Q5 Reaction Buffer Pack	6.0 ml

Revision History

REVISION #	DESCRIPTION	DATE
1.0		12/12
2.0		11/14
3.0		6/18
4.0	Update to new manual format	1/20