

Q5[®] High-Fidelity PCR Kit

NEB #E0555S/L

50/200 reactions

Version 4.0_1/20

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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)

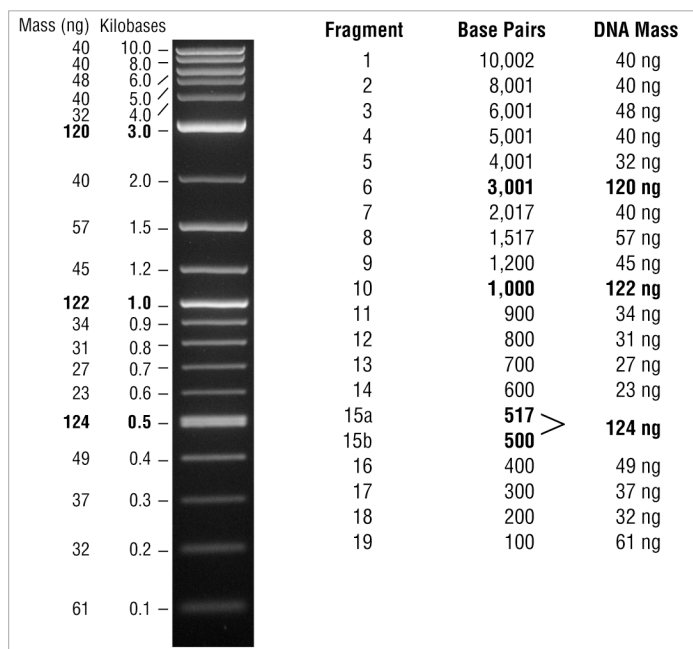
Quick-Load Purple 1 kb Plus DNA Ladder is a pre-mixed, ready-to-load molecular weight marker containing one tracking dye. Tracking dye does not leave a shadow under UV light.

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 MgCl₂.

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).



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 μ l reaction are as follows:

| DNA | AMOUNT |
|------------------|----------------|
| Genomic | 1 ng-1 μ g |
| 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 μ M in the reaction.
3. **Mg⁺⁺ and additives:**
The Q5 High-Fidelity Master Mix contains 2.0 μ M 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 μ M 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 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 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 $\leq 72^{\circ}\text{C}$ 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 ($3' \rightarrow 5'$ exo⁻) (NEB #M0212).

Troubleshooting Guide:

| PROBLEM | POSSIBLE CAUSE | SOLUTION(S) |
|------------------------|---------------------------------------|---|
| Sequence Errors | Suboptimal reaction conditions | <ul style="list-style-type: none"> Reduce number of cycles Decrease extension time |
| | Template DNA has been damaged | <ul style="list-style-type: none"> 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 | <ul style="list-style-type: none"> Clone into a non-expression vector Use a low copy number cloning vector |
| Incorrect Product Size | Incorrect annealing temperature | <ul style="list-style-type: none"> Recalculate primer T_m values using the NEB T_m calculator (www.neb.com/TmCalculator) |
| | Mispriming | <ul style="list-style-type: none"> Verify that primers have no additional complementary regions within the template DNA |
| | Nuclease contamination | <ul style="list-style-type: none"> Repeat reactions using fresh solutions |
| No Product | Incorrect annealing temperature | <ul style="list-style-type: none"> Recalculate primer T_m values using the NEB T_m calculator (www.neb.com/TmCalculator) Test an annealing temperature gradient, starting at 5°C below the lower T_m of the primer pair |
| | Poor primer design | <ul style="list-style-type: none"> 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 | <ul style="list-style-type: none"> Verify that oligos are complementary to proper target sequence |
| | Insufficient primer concentration | <ul style="list-style-type: none"> Verify that each primer is added to a final concentration of 0.5 μM |
| | Missing reaction component | <ul style="list-style-type: none"> Repeat reaction setup |
| | Poor template quality | <ul style="list-style-type: none"> Analyze DNA via gel electrophoresis before and after incubation with Mg⁺⁺ Check 260/280 ratio of DNA template |
| | Presence of inhibitor in reaction | <ul style="list-style-type: none"> Further purify starting template by alcohol precipitation, drop dialysis or commercial clean-up kit Decrease sample volume |
| | Insufficient number of cycles | <ul style="list-style-type: none"> Rerun the reaction with more cycles |

| PROBLEM | POSSIBLE CAUSE | SOLUTION(S) |
|-----------------------------------|--|---|
| No Product | Incorrect thermocycler programming | <ul style="list-style-type: none"> • Check program, verify times and temperatures |
| | Inconsistent thermocycler block temperature | <ul style="list-style-type: none"> • Test calibration of heating block |
| | Contamination of reaction tubes or solutions | <ul style="list-style-type: none"> • 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 | <ul style="list-style-type: none"> • 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 | <ul style="list-style-type: none"> • Increase annealing temperature |
| | Poor primer design | <ul style="list-style-type: none"> • 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 | <ul style="list-style-type: none"> • Verify that each primer is added to a final concentration of 0.5 μM |
| | Contamination with exogenous DNA | <ul style="list-style-type: none"> • 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 | <ul style="list-style-type: none"> • 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 μ l reaction containing 20 ng Genomic DNA with 1X Q5 High-Fidelity 2X Master Mix and 0.5 μ M of each primer result in the expected 7 kb product.

20 kb Lambda DNA PCR:

22 cycles of PCR amplification in a 50 μ l reaction containing 10 ng Lambda DNA with 1X Q5 High-Fidelity 2X Master Mix and 1.0 μ M 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 |

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