

PCR Reagents

POLYMERASES, NUCLEOTIDES, & DNA LADDERS





Unparalleled confidence.

For over 25 years, New England Biolabs has been committed to the development of innovative, high quality tools for your PCR, qPCR and related amplification technologies. Our product quality, enzyme expertise and outstanding technical support bring unparalleled confidence to your experiments. Find out more at nebper.com.

Featured Products Include:

- Luna® qPCR & RT-qPCR Reagents: for rapid, sensitive and precise detection of RNA, DNA and cDNA targets
- Q5 High-Fidelity DNA Polymerase: for robust, ultra high-fidelity PCR
- One Taq DNA Polymerase: for robust, routine PCR
- ProtoScript[®] II Reverse Transcriptase: for efficient reverse transcription
- Bst DNA Polymerases and WarmStart® LAMP Products: for robust isothermal amplification
- Exo-CIP Rapid PCR Cleanup Kit: for rapid degradation of PCR primers and dephosphorylation of dNTPs following amplification
- dNTPs: ultrapure solution sets and mixes for a variety of applications

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Related Resources at neb.com:

- Visit <u>LUNAqPCR.com</u> to learn more about the Luna portfolio of products for qPCR & RT-qPCR
- Visit www.neb.com/isoamp to find a wide range of tools for isothermal amplification, including Bst DNA Polymerases, WarmStart RTx, unique enzymes and master mixes supporting LAMP, SDA/NEAR, WGA/ MDA, RPA and other methods
- Visit <u>NEBrna.com</u> to find products supporting RNA workflows
- Visit <u>www.neb.com/supporting-molecular-diagnostics</u> to find amplification-based products for molecular diagnostic applications

PCR Polymerase Selection Chart

When choosing a polymerase for PCR, we recommend starting with OneTaq $^{\text{®}}$ or Q5 $^{\text{®}}$ DNA Polymerases (shown below in gold). Both offer robust amplification and can be used on a wide range of templates (routine, AT- and GC-rich). Q5 provides the benefit of maximum fidelity, and is also available in a formulation specifically optimized for next generation sequencing.

* indicates recommended choice for application

| | | | | | | | ★ indicates re | ecommended c | hoice for applica | ation |
|---------------------------|---|--------------------------------------|----------------------------------|---|----------------------|---|----------------|---|-------------------|------------------|
| | STAND | ARD PCR | HI | GH-FIDELITY | PCR | | 5 | SPECIALTY P | CR | |
| | | | | HEST ELITY | MODERATE FIDELITY | LONG Amplicons | d TOLEF | U Rance | BLOOD DIRECT PCR | |
| June 1 | One <i>Taq®/</i> One <i>Taq</i> Hot Start | <i>Taq </i> Hot Start <i>Taq</i> | Q5 [®] /Q5 Hot Start | Phusion ^{®(4)} / Phusion ⁽⁴⁾ Flex | Vent®/ Deep Vent™ | LongAmp [®] / LongAmp Hot Start <i>Taq</i> | Q5U® | Epimark [®] Hot Start <i>Taq</i> | Q5 Blood | Hemo KlenTaq® |
| PROPERTIES | | | | | | | | | | |
| Fidelity vs. <i>Taq</i> | 2X | 1X | ~280X ⁽²⁾ | > 39X | 5-6X | 2X | ND | 1X | ND | ND |
| Amplicon Size | < 6 kb | ≤ 5 kb | ≤ 20 kb | ≤ 20 kb | ≤ 6 kb | ≤ 30 kb | app-specific | ≤ 1 kb | ≤ 7.5 kb | ≤ 2 kb |
| Extension Time | 1 kb/min | 1 kb/min | 6 kb/min | 4 kb/min | 1 kb/min | 1.2 kb/min | 2 kb/min | 1 kb/min | 2-4 kb/min | 0.5 kb/min |
| Resulting Ends | 3´ A/Blunt | 3´ A | Blunt | Blunt | Blunt | 3´ A/Blunt | Blunt | 3´ A | Blunt | 3´ A |
| 3'→5' exo | Yes | No | Yes | Yes | Yes | Yes | Yes | No | Yes | No |
| 5'→3' exo | Yes | Yes | No | No | No | Yes | No | Yes | No | No |
| Units/50 µl Reaction | 1.25 | 1.25 | 1.0 | 1.0 | 0.5-1.0 | 5.0 | 1.0 | 1.25 | N/A | N/A |
| Annealing Temperature | Tm ⁻ 5 | Tm⁻5 | Tm+3 | Tm+3 | Tm ⁻ 5 | Tm⁻5 | Tm+3 | Tm ⁻ 5 | Tm+3 | Tm⁻5 |
| APPLICATIONS | | | | | | | | | | |
| Routine PCR | * | • | • | • | • | • | | | | |
| Colony PCR | * | • | | | | | | | | |
| Enhanced Fidelity | • | | * | • | • | • | • | | • | |
| High Fidelity | | | * | • | | | • | | • | |
| High Yield | * | • | * | • | | | | | • | |
| Fast | | | * | • | | | | | • | |
| Long Amplicon | | | * | • | | * | | | | |
| GC-rich Targets | * | | * | | • | • | | | • | |
| AT-rich Targets | * | • | * | • | | • | * | • | | |
| High Throughput | • | • | • | • | | | * | • | | |
| Multiplex PCR | • | ★ ⁽¹⁾ | • | • | | | | | • | |
| Extraction-free PCR | | | | | | | | | * | • |
| DNA Labeling | | * | | | | | | | | |
| Site-directed Mutagenesis | | | * | • | | | | | | |
| Carryover Prevention | | | | | | | * | • | | |
| USER® Cloning | | | | | | | * | • | | |
| NGS APPLICATIONS | | | | | | | | | | |
| NGS Library Amplification | | | ★(3) | • | | | ★(6) | ★(5) | | |
| FORMATS | | | | | | | | | | |
| Hot Start Available | • | • | • | • | | • | • | • | • | |
| Kit | | • | • | • | | • | | | | |
| Master Mix Available | • | • | • | • | | • | | | • | |
| D: 10.11 II | | | | | | | | | | |

(1) Use Multiplex PCR 5X Master Mix

Direct Gel Loading

- (2) Due to the very low frequency of misincorporation events being measured, the error rate of high-fidelity enzymes like Q5 is challenging to measure in a statistically significant manner. We continue to investigate improved assays to characterize Q5's very low error rate to ensure that we present the most robust accurate fidelity data possible (Popatov, V. and Ong, J.L. (2017) PLoS One, 12(1):e0169774. doi 10.1371/journal. pone. 0169774).
- (3) Use NEBNext High-Fidelity 2X PCR Master Mix.
- (4) Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific.
- Use NEBNext Enzymatic Methyl-seq Kit (EM-seq[™]) for Illumina.
- (6) Use NEBNext Q5U Master Mix.





Q5[®] High-Fidelity DNA Polymerase Q5 Hot Start High-Fidelity DNA Polymerase

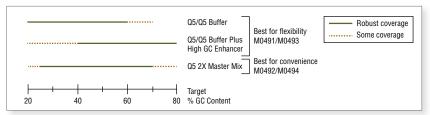
Q5 High-Fidelity DNA Polymerase sets a new standard for both fidelity and performance. With the highest fidelity amplification available (~280X higher than *Taq* and > 5X higher than Thermo Scientific® Phusion®), Q5 DNA Polymerase results in ultra-low error rates. Q5 DNA Polymerase is composed of a novel polymerase that is fused to the processivity-enhancing Sso7d DNA binding domain, improving speed, fidelity and reliability of performance.

In contrast to chemically-modified or antibody-based hot start polymerases, Q5 Hot Start utilizes a unique synthetic aptamer. This structure binds to the polymerase through non-covalent interactions, blocking activity during the reaction setup. The polymerase is activated during normal cycling conditions, allowing reactions to be set up at room temperature. Q5 Hot Start does not require a separate high temperature activation step, shortening reaction times and increasing ease-of-use. Q5 Hot Start is an ideal choice for high specificity amplification and provides robust amplification of a wide variety of amplicons, regardless of GC content.

Also available: Q5 High-Fidelity DNA Polymerase optimized for NGS applications. Visit NEBNext.com for details.

| Q5 High-Fidelity DNA Polymerase | <u>M0491S/L</u> |
|--|-----------------|
| Q5 High-Fidelity 2X Master Mix | <u>M0492S/L</u> |
| Q5 Hot Start High-Fidelity DNA Polymerase | <u>M0493S/L</u> |
| Q5 Hot Start High-Fidelity 2X Master Mix | <u>M0494S/L</u> |
| NEW Q5 Blood Direct 2X Master Mix | <u>M0500S/L</u> |
| Q5 High-Fidelity PCR Kit | <u>E0555S/L</u> |
| NEBNext® High-Fidelity 2X PCR Master Mix | <u>M0541S/L</u> |
| NEBNext Ultra™ II Q5 Master Mix | <u>M0544S/L</u> |
| Q5U Hot Start High-Fidelity DNA Polymerase | <u>M0515S/L</u> |
| NEBNext Q5U Master Mix | <u>M0597S/L</u> |
| | |

Q5 DNA Polymerases offer exceptional coverage over the entire range of GC composition



The stand-alone enzyme comes with a reaction buffer that supports robust amplification of high AT to routine targets. Addition of the High GC Enhancer allows amplification of GC rich and difficult targets. For added convenience, the master mix formats allow robust amplification of a broad range of targets with a single formulation.

Fidelity at its finest.



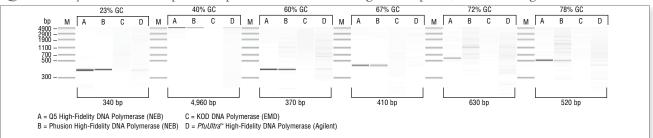
| Extension Data | 6 l/h/min |
|----------------------------|-------------------------------|
| Extension Rate | |
| Amplicon Size | |
| Fidelity | ~280X <i>Taq</i> |
| Units/50 µl rxn | 1 unit |
| Resulting Ends | |
| 3´→5´ Exonuclease Activity | |
| 5´→3´ Exonuclease Activity | |
| Supplied Buffer | |
| Supplied Enhancer | Q5 High GC Enhancer |
| Compatible w/Other Buffers | with Reduced Activity Profile |
| Product Formats | |
| Hot Start Available | Yes |
| | No |
| Master Mix Available | |
| PCR Kit Available | |
| | |
| NGS Version Available | |
| Applications | |
| High-Fidelity PCR | Yes |
| Difficult PCR | |
| High GC PCR | |
| T/A, U/A Cloning | |
| | |
| Colony PCR | |



Learn how Q5 can be used in multiplex PCR in our application note at Q5PCR.com

Blunt Cloning Yes

Q5 DNA Polymerase offers superior amplification for a wide range of templates, even with high GC content



Amplification of a variety of human genomic amplicons from low to high GC content demonstrates the broad performance of Q5 High-Fidelity DNA Polymerase. All reactions were conducted using 20 ng of input template and included 30 cycles of amplification. Results were visualized by microfluidic LabChip® analysis. Competitor polymerases were cycled according to manufacturer's recommendations. For the final three amplicons, GC Buffers or enhancers were used when supplied with the polymerase.

Comparison of high-fidelity polymerases

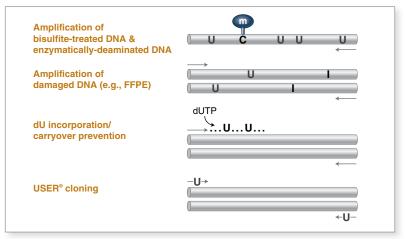
| PRODUCT NAME (Supplier) | POLYMERASE FIDELITY (Reported by supplier) | MAXIMUM AMPLICON LENGTH ⁵ | EXTENSION TIME ⁵ (For simple templates ⁴) | EXTENSION TIME ⁵ (For complex templates ⁴) | |
|---|--|---|---|--|--|
| Q5 High-Fidelity DNA Polymerase (NEB) | ~280X <i>Taq</i> ¹ | 20 kb simple; 10 kb complex | 10 s/kb | 10 s/kb (< 1 kb) 20–30 s/kb (> 1 kb) | |
| Phusion High-Fidelity DNA Polymerase* (NEB) | 39X <i>Taq</i> ¹ | 20 kb simple; 10 kb complex | 15 s/kb 30 s/kb | | |
| AccuPrime™ <i>Pfx</i> (Life) | 26X Taq ² | 12 kb³ | 60 |) s/kb³ | |
| <i>PfuUltra</i> ™ II Fusion HS (Agilent) | 20X <i>Taq</i> ² | 19 kb³ | 15 s/kb (< 10 kb³) 30 s/kb (> 10 kb³) | | |
| PfuUltra High-Fidelity DNA Polymerase (Agilent) | 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 | | 60 s/kb (< 10 kb) 120 s/kb (> 10 kb) | 60 s/kb (< 6 kb) 120 s/kb (> 6 kb) | |
| KOD DNA Polymerase (EMD) | IA Polymerase 12X <i>Taq</i> ¹ 6 kb simple; 2 kb complex 10–20 s/kb | | 30-60 s/kb | | |
| Platinum® <i>Taq</i> HiFi (Life) 6X <i>Taq</i> ² 20 kb ³ 60 s/kb ³ | | | |) s/kb³ | |

We continue to investigate improved assays to characterize Q5's very low error rate to ensure that we present the most accurate fidelity data possible (Potapov, V. and Ong, J.L. (2017) PLoS ONE. 12(1): e0169774.).

Q5U® Hot Start High-Fidelity DNA Polymerase

Q5U Hot Start High-Fidelity DNA Polymerase is a modified version of Q5 High-Fidelity DNA Polymerase, a novel thermostable DNA polymerase that possesses 3′ to 5′ exonuclease activity, and is fused to a processivity-enhancing Sso7d domain. Q5U contains a mutation in the uracil-binding pocket enables the ability to read and amplify templates containing uracil and inosine bases. Q5U contains a unique aptamer selected for polymerase inhibition at room temperature and optimal amplification during typical PCR conditions.

Common applications enabled by Q5U Hot Start High-Fidelity DNA Polymerase



Archaeal family B-type polymerases can incorporate/tolerate a variety of modified nucleotides but will stall upon encountering uracil and inosine residues. Q5U Hot Start High-Fidelity DNA Polymerase is a modified Q5 High-Fidelity DNA polymerase, which efficiently incorporates dUTP and amplifies uracil-containing templates.

² PCR-based mutation screening in lacZ (NEB), lacI (Agilent) or rpsL (Life)

^{*} Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific.

³ Template not specified.

⁴ Simple templates include plasmid, viral and E. coli genomic DNA. Complex templates include plant, human and other mammalian genomic DNA.

⁵ Values provided by individual manufacturers.



One *Taq*[®] DNA Polymerase One *Taq* Hot Start DNA Polymerase

An optimized blend of Taq and Deep Vent DNA polymerases, One Taq and One Taq Hot Start DNA Polymerases offer robust amplification across a wide range of templates. The $3' \rightarrow 5'$ exonuclease activity of Deep Vent DNA Polymerase increases the fidelity and robustness of Taq. Additionally, One Taq Reaction Buffers and High GC Enhancer have been formulated for robust yields with minimal optimization, regardless of a template's GC content.

One *Taq* DNA Polymerase is supplied with two 5X buffers (Standard and GC), as well as a High GC Enhancer solution. For most routine, AT- rich or complex amplicons with up to ~65% GC content, One *Taq* Standard Reaction Buffer provides robust amplification. For GC-rich amplicons, the One *Taq* GC Reaction Buffer can improve both performance and yield. For particularly high GC (> 65%) or difficult amplicons, the One *Taq* High GC Enhancer can be added to reactions containing One *Taq* GC Buffer. These formulations ensure maximum performance for routine, AT- or GC-rich amplicons.

Master Mix Formulations

In addition to standalone enzymes, both One Taq and One Taq Hot Start DNA Polymerases are available in master mix and Quick-Load® master mix formats. Master mix formulations include dNTPs, MgCl₂ and other buffers and stabilizers. The Quick-Load master mix formulations also include two tracking dyes for use with downstream visualization (i.e., agarose gels). With these convenient formats, the addition of primers and template are all that is required for robust amplification.

| One Taq DNA Polymerase | $\underline{\text{M0480S/L/X}}$ |
|---|---------------------------------|
| One Taq 2X Master Mix with Standard Buffer | <u>M0482S/L</u> |
| One Taq Quick-Load 2X Master Mix with Standard Buffer | <u>M0486S/L</u> |
| One Taq Quick-Load DNA Polymerase | M0509S/L |

One Taq Buffer Recommendations

| AMPLICON % GC BUFFER | RECOMMENDED DEFAULT BUFFER | OPTIMIZATION Notes |
|-------------------------|---|--|
| < 50% GC | One <i>Taq</i> Standard Reaction Buffer | Adjust annealing temperature, primer/ template concentration, etc. if needed. |
| 50-65% GC | One <i>Taq</i> Standard Reaction Buffer | One <i>Taq</i> GC Reaction Buffer can be used to enhance performance of difficult amplicons. |
| > 65% GC | One <i>Taq</i> GC Reaction Buffer | One <i>Taq</i> GC Reaction Buffer with 10–20% One <i>Taq</i> High GC Enhancer can be used to enhance performance of difficult amplicons. |



| Extension Data | 1 l/h/min |
|--------------------------------------|--|
| Extension Rate | • |
| Amplicon Size | |
| Fidelity | |
| Units/50 µl rxn | |
| Resulting Ends | 3´ A/Blunt |
| 3´→5´ Exonuclease Activity | Yes |
| 5´→3´ Exonuclease Activity | Yes |
| Supplied Buffer | One <i>Taq</i> Std Rxn Buffer, One <i>Taq</i> GC Rxn Buffer |
| Supplied Enhancer | One Taq High GC Enhancer |
| Compatible w/Other Buffers | with Reduced Activity Profile |
| Product Formats Hot Start Available | Vac |
| | |
| - Activation Required | |
| Master Mix Available | |
| Direct Gel-loading Available | |
| PCR Kit Available | INO |
| Applications | |
| Routine PCR | Yes |
| SNP Detection | Yes |
| T/A, U/A Cloning | Yes |
| Colony PCR | Yes |
| | |





One *Taq* Hot Start DNA Polymerase allows room temperature reaction setup with no separate activation step

In contrast to chemically-modified or antibody-based hot start polymerases, NEB's One *Taq* Hot Start utilizes aptamer technology. This aptamer/inhibitor binds to the polymerase through non-covalent interactions, blocking polymerase activity at temperatures below 45°C. The polymerase is activated during normal cycling conditions, allowing reactions to be set up at room temperature. One *Taq* Hot Start DNA Polymerase does not require a separate high temperature incubation step to activate the enzyme and can be used in typical *Taq*-based cycling protocols. This ultimately shortens reaction times and increases ease of use.

| One Taq Hot Start DNA Polymerase | M0481S/L/X |
|---|-----------------|
| One Taq Hot Start 2X Master Mix with Standard Buffer | <u>M0484S/L</u> |
| One Taq Hot Start 2X Master Mix with GC Buffer | <u>M0485S/L</u> |
| One Taq Hot Start Quick-Load 2X Master Mix with Standard Buffer | <u>M0488S/L</u> |
| One Tag Hot Start Quick-Load 2X Master Mix with GC Buffer | M0489S/L |



To learn how One *Taq* can be used in colony PCR, download the application note at www.neb.com/OneTaq

Comparison of One Taq Hot Start DNA Polymerase to other commercially available hot start polymerases.

| Polymerase | Additives | 55 | 65 | 66 | 5% 67 | 78 | 79 | |
|--|--|-------------------------|---------------------------------|---------------|---------------|--------------|-----------|--|
| One <i>Taq</i> ® DNA Polymerase (NEB) | None* | • | • | | • | • | • | |
| OneTaq HotStart DNA Polymerase (NEB) | None* | • | • | | • | | • | |
| AmpliTaq Gold® 360 DNA Polymerase (Thermo Fisher) | None 360 GC Enhancer | • | | • | | • | | |
| DreamTaq® Hot Start DNA Polymerase (Thermo Fisher) | (Not provided) | • | | • | | • | • | |
| FastStart™ Taq DNA Polymerase (Roche) | None GC-RICH solution | • | | • | | • | | |
| GoTaq® G2 Hot Start Polymerase (Promega) | (Not provided) | • | | • | | • | • | |
| GoTaq Hot Start Polymerase (Promega) | (Not provided) | • | | • | | • | • | |
| HotStarTaq® DNA Polymerase (Qiagen) | Q-Solution None | • | | | | | • | |
| HotStarTaq Plus DNA Polymerase (Qiagen) | Q-Solution None | • | | | | • | • | |
| iTaq™ DNA polymerase (Bio-Rad) | (Not provided) | • | | | | • | | |
| JumpStart™ Taq DNA Polymerase (Sigma) | None | • | | | | | | |
| Platinum™ II Taq Hot-Start DNA Polymerase (Thermo Fisher) | Platinum GC Enhancer None | • | | • | • | | | |
| Platinum Taq DNA Polymerse High Fidelity (Thermo Fisher) | (Not provided) | | | | | • | • | |
| Platinum Taq DNA Polymerase (Thermo Fisher) | None KB Extender | • | | | | | | |
| Ex Taq® DNA Polymerase, hot-start version (TaKaRa) | (Not provided) | | | | | | | |
| Titanium™ Taq DNA Polymerase (TaKaRa) | (Not provided) | | • | • | | | | |
| Yield (ng/ul) 0.0 1.0 2.0 3.0 4.0 ≥5.0 | * One Taq prod Reaction Buff table. For oth GC enhancer | er. The GC er products, | reaction buffe amplification | er was used t | o amplify the | targets show | vn in the | |



Phusion[®] High-Fidelity DNA Polymerase

DNA polymerases with high fidelity are important for applications in which the DNA sequence needs to be correct after amplification. Manufactured and quality controlled at New England Biolabs, Thermo Scientific Phusion High-Fidelity DNA Polymerase offers both high fidelity and robust performance, and thus can be used for all PCR applications. Its unique structure, a novel *Pyrococcus*-like enzyme fused with a processivity-enhancing domain, increases fidelity and speed. Product selection includes a standalone enzyme, master mix and kit format, as well as a choice of reaction buffers for amplification of difficult templates. Phusion Hot Start Flex DNA Polymerase is available as standalone enzyme or in a master mix format and enables high specificity amplification of a broad range of templates with the flexibility of room temperature setup.

| Phusion High-Fidelity DNA Polymerase | . <u>M0530S/L</u> |
|---|-------------------|
| Phusion High-Fidelity PCR Kit | <u>E0553S/L</u> |
| Phusion High-Fidelity PCR Master Mix with HF Buffer | . <u>M0531S/L</u> |
| Phusion High-Fidelity PCR Master Mix with GC Buffer | . <u>M0532S/L</u> |
| Phusion Hot Start Flex | . <u>M0535S/L</u> |
| Phusion Hot Start Flex 2X Master Mix | . M0536S/L |

Phusion DNA Polymerase generates amplicons with high yield and much shorter extension times

| | Phu | ısion 1 1 ι | from unit) | NEB | | | gh Fid (2.5 | | | | N | | ed KC unit) | D | |
|---|-----|----------------|---------------|-----|---|---|----------------|-----|-----|---|---|-----|----------------|-----|-----|
| M | 1 | 1.5 | 3.8 | 7.6 | M | 1 | 1.5 | 3.8 | 7.6 | M | 1 | 1.5 | 3.8 | 7.6 | min |
| J | | | | 100 | _ | | | | | | | | 8.8 | | |
| | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |
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A 3.8 kb fragment was amplified from 50 ng of Jurkat gDNA using different polymerases. Reactions were carried out according to the manufacturer's recommended conditions. Extension times are indicated (in minutes). Ladder M is a 1 kb DNA Ladder (NEB #N3232).

Phusion Buffer Selection Chart

| CHOICE OF BUFFER | APPLICATION | NEB # |
|------------------------|---|--------|
| Phusion HF Buffer Pack | Default buffer for high-fidelity amplification | B0518S |
| Phusion GC Buffer Pack | For long, difficult or GC-rich templates (when HF buffer fails) | B0519S |

Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific.

| 4 kb/min |
|---|
| ≤ 20 kb |
| 39X <i>Taq</i> |
| 1 units |
| Blunt |
| Yes |
| No |
| . 5X Phusion HF Buffer, 5X Phusion GC Buffer |
| 100% DMS0 |
| No |
| |
| Yes |
| No |
| Yes |
| Yes |
| |
| Yes |
| No |
| No |
| Yes |
| |



High-fidelity polymerases benefit from a Tm⁺3 annealing temperature. Use the NEB Tm Calculator to ensure successful PCR at **TmCalculator.neb.com**.



Taq DNA Polymerase

For routine amplification, where cost per reaction and yield are the priorities, Taq DNA Polymerase is the industry standard. NEB provides high quality recombinant Taq at an exceptional value. To accommodate a variety of PCR applications, Taq is available with different reaction buffers. Standard Taq Buffer is designed to support existing PCR platforms and is an ideal choice for DHPLC and high-throughput applications. ThermoPol Buffer is formulated to promote high product yields, even under demanding conditions.

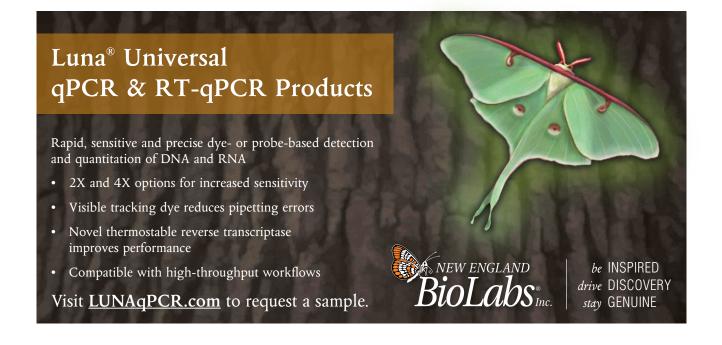
| Taq DNA Polymerase with Standard Taq Buffer | <u>M0273S/L/X/E</u> |
|---|---------------------|
| Taq DNA Polymerase with Standard Taq (Mg-free) Buffer | <u>M0320S/L</u> |
| Taq DNA Polymerase with ThermoPol Buffer | <u>M0267S/L/X/E</u> |
| Taq PCR Kit | <u>E5000S</u> |
| Taq 5X Master Mix | <u>M0285L</u> |
| Taq 2X Master Mix | <u>M0270L</u> |
| Quick-Load Taq 2X Master Mix | <u>M0271L</u> |
| Hot Start Taq DNA Polymerase | <u>M0495S/L</u> |
| Hot Start Taq 2X Master Mix | <u>M0496S/L</u> |

Looking for a hot start *Taq* for use in molecular diagnostics? Contact us at custom@neb.com

Taq Buffer Selection Chart

| CHOICE OF BUFFER | | MG-CONTROL | NEB# |
|------------------|--|--|--------------|
| | Standard <i>Taq</i> Reaction Buffer: Detergent-free and designed to be | <i>Taq</i> with Standard <i>Taq</i> Buffer | M0273S/L/X |
| | compatible with existing assay systems | Taq with Standard Taq (Mg-free) Buffer | M0320S/L |
| | ThermoPol Buffer: Designed to optimize yields and specificity | Taq with ThermoPol Buffer | M0267S/L/X/E |

| DLYMERASE DETAILS | |
|---|---|
| Extension Rate | 1 kb/min |
| Amplicon Size | ≤5 kb |
| Units/50 µl rxn | 1.25 units |
| Resulting Ends | 3´ A |
| 3´→5´ Exonuclease Activity | No |
| 5´→3´ Exonuclease Activity | Yes |
| Supplied BufferStandard or Therm | <i>Taq</i> Rxn Buffer, oPol Rxn Buffer |
| Compatible w/Other Tag Buffers | Vρα |
| Compatible w/Other <i>Taq</i> Buffers | |
| Product Formats | |
| Product Formats Hot Start Available | Yes |
| Product Formats Hot Start Available - Activation Required. | Yes |
| Product Formats Hot Start Available - Activation Required. Master Mix Available | YesYes |
| Product Formats Hot Start Available Activation Required. Master Mix Available. Direct Gel-loading Available. | YesYes |
| Product Formats Hot Start Available Activation Required. Master Mix Available. Direct Gel-loading Available. PCR Kit Available. | YesYes |
| Product Formats Hot Start Available Activation Required. Master Mix Available. Direct Gel-loading Available. | YesYes |
| Product Formats Hot Start Available Activation Required. Master Mix Available. Direct Gel-loading Available. PCR Kit Available. | YesYesYesYes |
| Product Formats Hot Start Available - Activation Required. Master Mix Available. Direct Gel-loading Available. PCR Kit Available. Applications | Yes No Yes Yes Yes Yes |
| Product Formats Hot Start Available - Activation Required. Master Mix Available. Direct Gel-loading Available. PCR Kit Available. Applications Routine PCR. | Yes No Yes Yes Yes Yes Yes Yes |



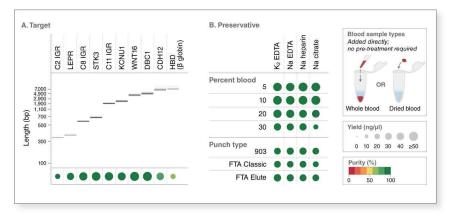


Q5® Blood Direct 2X Master Mix

Q5 Blood Direct 2X Master Mix can amplify a wide variety of targets directly from dried blood spots or up to 30% whole human blood, skipping DNA purification. The master mix includes Q5 Hot Start High-Fidelity DNA Polymerase and dNTPs in an optimized buffer with increased resistance to inhibitors in blood, anti-coagulants, and chemicals on filter papers. It is capable of amplifying products up to 7.5 kb from human whole blood cells preserved with sodium EDTA, potassium EDTA, sodium citrate and sodium heparin as well samples stored on common preservative filter papers.

Q5 Blood Direct 2X Master Mix M0500S/L

Q5 Blood Direct 2X Master Mix enables robust amplification direct from whole or dried blood



PCR was performed using Q5 Blood Direct 2X Master Mix under standard recommended conditions with 35 cycles of amplification. Yield and purity were quantitated by microfluidic LabChip® analysis and are indicated by dot size and color, respectively, with a large, dark green dot representing the strongest performance. A. Amplification of a variety of human genomic amplicons, 0.3 to 7.5 kb in length, from 10% EDTA-preserved human whole blood. Results are shown as both a virtual gel (top) and corresponding dot plot (bottom). Q5 Blood Direct 2X Master Mix performs well across a broad range of amplicon sizes. B. Amplification of a 604 bp human genomic amplicon from whole blood (top) or blood dried on filters (bottom). Human whole blood comprised 5-30% of the total reaction volume (50 µl) as indicated. Untreated 1 mm punches from dried blood spots were added directly to 25 µl reactions (one punch per reaction), even where pre-treatment of the punch was recommended by the manufacturer. Q5 Blood Direct 2X Master Mix shows broad tolerance to varying blood volumes, preservatives and punch types.

EpiMark® Hot Start *Taq* DNA Polymerase for bisulfite sequencing

EpiMark Hot Start *Taq* DNA Polymerase is a mixture of *Taq* DNA Polymerase and a temperature sensitive, aptamer-based inhibitor. This inhibitor binds reversibly to the enzyme, inhibiting polymerase activity below 45°C, but releases the enzyme during normal PCR cycling conditions. With a reaction buffer that has been optimized for AT-rich templates, EpiMark Hot Start *Taq* is an excellent choice for bisulfite-treated DNA.

POLYMERASE DETAILS

| Extension Rate | 2-4 | kb/min |
|----------------------------|-----|----------|
| Amplicon Size | : | ≤ 7.5 kb |
| Units/50 µl rxn | | N/A |
| Resulting Ends | | . Blunt |
| 3´→5´ Exonuclease Activity | | Yes |
| 5´→3´ Exonuclease Activity | | No |
| Supplied Buffer | | N/A |
| Compatible w/Other Buffers | | N/A |
| | | |

Applications

| Extraction-free PCR | | | | | | | | | | .Yes |
|---------------------|------|--|--|--|--|--|--|--|--|------|
| T/A, U/A Cloning | | | | | | | | | | . No |
| Cloning PCR | | | | | | | | | | . No |

ADVANTAGES

- Ideal for use on bisulfite-converted DNA and AT-rich templates
- Specially formulated reaction buffer system
- Aptamer-based hot start technology avoids nonspecific amplification and enables room temperature reaction setup

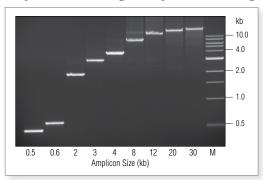


LongAmp® Taq enables extension of longer amplicons

An optimized blend of *Taq* and Deep Vent DNA Polymerases, LongAmp *Taq* DNA Polymerase enables amplification of up to 30 kb PCR products with a fidelity higher than *Taq* DNA Polymerase alone.

| LongAmp Taq DNA Polymerase | M0323S/L |
|--------------------------------------|---------------|
| LongAmp Taq PCR Kit | <u>E5200S</u> |
| LongAmp Taq 2X Master Mix | M0287S/L |
| LongAmp Hot Start Taq DNA Polymerase | M0534S/L |
| LongAmp Hot Start Taq 2X Master Mix | M0533S/L |

Amplification of longer templates with LongAmp Taq



Amplification of specific sequences from human genomic DNA using LongAmp Taq DNA Polymerase. Amplicon sizes are indicated below gel. Marker M is NEB 1 kb DNA Ladder (NEB #N3232).

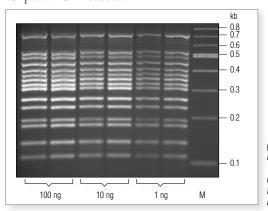
POLYMERASE DETAILS Amplicon Size≤ 30 kb Fidelity 2X Tag Units/50 µl rxn 5 units Resulting Ends3´ A/Blunt Supplied Buffer..... LongAmp or *Taq* Rxn Buffer Compatible w/Other Tag Buffers . . . with Reduced Activity Profile **Product Formats** Hot Start Available Yes - Activation Required No Direct Gel-loading Available. Yes PCR Kit Available Yes **Applications** Long Amplicons Yes Routine PCR Yes T/A, U/A Cloning Yes

Multiplex PCR 5X Master Mix for multiple templates

Multiplex PCR can simultaneously detect two or more products in a single reaction. Multiplex PCR can also be used for semi-quantitative gene expression analysis using cDNA templates. The NEB Multiplex PCR 5X Master Mix is an easy-to-use solution featuring high quality recombinant *Taq* DNA Polymerase. The mix is optimized for high yield and performance. Its performance is illustrated below in a 15-plex PCR reaction using human genomic DNA. The 5X formulation allows maximal flexibility for input of custom primers and template DNAs.

Multiplex PCR 5X Master Mix M0284S

15-plex PCR reaction



15-plex PCR using varying amounts of human genomic DNA. 1X Multiplex PCR 5X Master Mix was used with 0.15 µM of each primer. The cycling conditions were 95°C for 1 minute 35 cycles of 95°C for 20 seconds, 60°C for 1 minute and 68°C for 2 minutes. Marker M is the 1 kb Plus DNA Ladder (NEB #N3200).

ADVANTAGES

- Simultaneously detect two or more products in a single reaction
- Enhanced buffer formulation for optimal performance
- 5X master mix format for easy reaction setup just add template DNA and primers



PreCR® Repair Mix

The PreCR Repair Mix is a cocktail of enzymes formulated to repair damaged DNA *in vitro* prior to PCR. The repair pre-treatment can be applied to techniques such as whole genome amplification, DNA sequencing and microarray analysis.

The PreCR Repair Mix can repair a wide range of damaged DNA, resulting from exposure to heat, low pH, oxygen, and/or UV light. The lesions repaired by the PreCR Repair Mix do not include all possible types of damage. For example, it cannot repair DNA crosslinks, such as those that occur during exposure to formalin, nor can the mix effectively repair highly fragmented DNA.

PreCR Repair Mix <u>M0309S/L</u>

For repair of DNA prior to next generation sequencing library preparation, we recommend the NEBNext FFPE DNA Repair Mix (NEB #M6630). Visit NEBNext.com to learn more.

ADVANTAGES

- Specific Treats damaged DNA without harming template
- Versatile Can be used in conjunction with any thermophilic polymerase
- Convenient PCR can be done directly on repair reaction
- Flexible Suitable for PCR, microarrays and other DNA technologies

Exo-CIP™ Rapid PCR Cleanup Kit

The Exo-CIP Rapid PCR Cleanup Kit contains optimized formulations of thermolabile Exonuclease I and thermolabile Calf Intestinal Phosphatase and is used to rapidly degrade residual PCR primers and dephosphorylate excess dNTPs after amplification. Degradation occurs in only 4 minutes at 37°C, and is immediately followed by rapid inactivation of the enzymes by heating for 1 minute at at 80°C. In just 5 minutes, the PCR product is ready for downstream analysis such as Sanger sequencing, SNP detection, or library preparation for NGS. The Exo-CIP Rapid PCR Cleanup Kit is compatible with all commonly-used reaction buffers.

Exo-CIP Rapid PCR Cleanup Kit <u>E1050S/L</u>

ADVANTAGES

- 5 minute protocol for enzymatic cleanup of primers and dNTPs
- · Improves sequencing results, allowing longer reads

Monarch® PCR & DNA Cleanup Kit (5 μg)

The Monarch PCR & DNA Cleanup Kit rapidly and reliably purifies up to 5 μ g of concentrated, high-quality DNA from PCR and other enzymatic reactions. The kit utilizes a bind/wash/elute workflow with minimal incubation and spin times. The columns ensure zero buffer retention and no carryover of contaminants, enabling elution of sample in volumes as low as 6 μ l. The buffers provided have been optimized, and do not require monitoring of pH. Eluted DNA is ready for use in restriction digests, DNA sequencing, ligation and other enzymatic manipulations. The protocol can also be modified to enable the purification of smaller DNA fragments, including oligonucleotides and ssDNA.

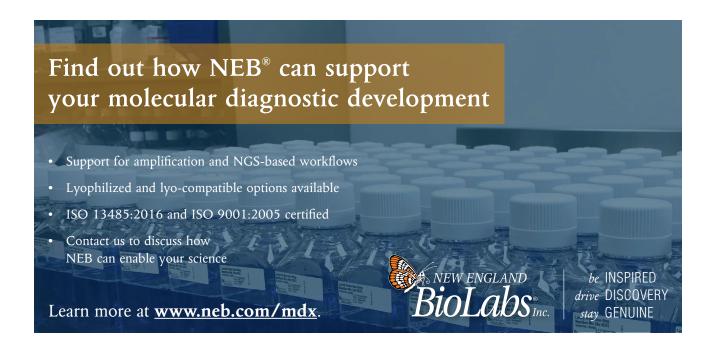
ADVANTAGES

- Elute in as little as 6 μl
- Prevent buffer retention and salt carryover with optimized column design
- Purify oligos and other small DNA fragments with simple protocol modification
- · Save time with fast, user-friendly protocols
- Designed with sustainability in mind



Nucleotide Solutions

| PRODUCT NAME | DESCRIPTION | NEB # |
|-------------------------------------|--|-----------------|
| Deoxynucleotide (dNTP) Solution Set | The Deoxynucleotide Solution Set contains four separate 100 mM solutions of ultrapure nucleotides (dATP, dCTP, dGTP, and dTTP). | <u>N0446S</u> |
| Deoxynucleotide (dNTP) Solution Mix | The Deoxynucleotide Solution Mix is an equimolar mixture of ultrapure dATP, dCTP, dGTP, and dTTP. Each nucleotide is present at a concentration of 10 mM in the mixture for a total dNTP concentration of 40 mM. | <u>N0447S/L</u> |
| 7-deaza-dGTP* | A useful additive for PCR of GC-rich templates; contains a 5 mM solution of 7-deaza-GTP as a dilithium salt. *licensed from Roche Diagnostics GmbH | <u>N0445S/L</u> |
| Acyclonucleotide Set | Acyclonucleotide Set contains four separate tubes of acyNTPs (acyATP, acyCTP, acyGTP and acyTTP). | <u>N0460S</u> |
| dATP Solution | Contains 0.25 ml of 100 mM ultrapure dATP. | <u>N0440S</u> |
| Ribonucleotide Solution Set | Ribonucleotide Solution Set consists of four separate 100 mM solutions of ATP, GTP, CTP and UTP. | <u>N0450S/L</u> |
| Ribonucleotide Solution Mix | The Ribonucleotide Solution Mix is an equimolar mixture of ribonucleotide triphosphates (rATP, rCTP, rGTP and rUTP). Each is supplied at a concentration of 80 mM for a total concentration of 320 mM. | N0466S/L |

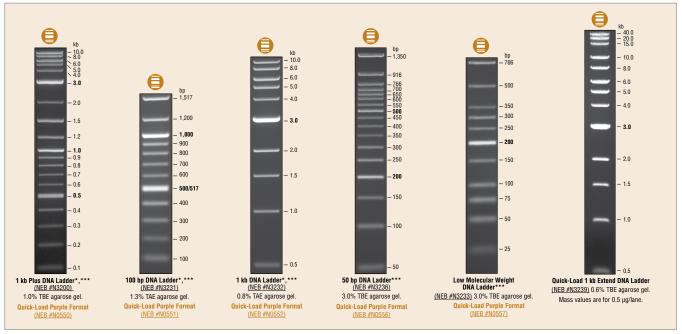




DNA Analysis

Agarose-gel electrophoresis is the standard method used for separation, identification and purification of DNA fragments. DNA is visualized on a gel after soaking or pre-casting the gel with a visualization dye, such as Ethidium Bromide, which is a DNA intercalating agent that fluoresces under UV illumination. DNA markers and ladders are composed of DNA fragments of known sizes and masses which are used as a reference to determine the size and relative mass of the DNA of interest. Bands are visible under UV illumination or under blue light illumination, depending on the visualization dye used. DNA markers and DNA samples have to be combined with loading dyes to give them density in the wells and to track the migration on the gel; some of NEB's ladders come pre-mixed with loading dye for convenience.

Quick-Load and Quick-Load Purple DNA Ladders

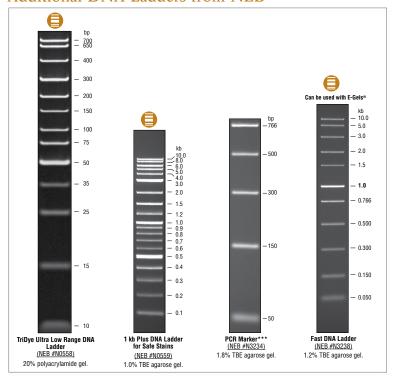


^{*} Available in Quick-Load® and TriDye™ formats



^{***} Free Loading Dye included

Additional DNA Ladders from NEB



- Sharp, crisp bands
- · Excellent quality and value
- Convenient 1 kb Plus DNA Ladder available in a variety of formats, including one specifically optimized for safe stains (e.g., GelRed® and SYBR® Safe)
- TriDye Ultra Low Range DNA Ladder ranges as low as 10 bp and is suitable for polyacrylamide gels
- For help with choosing a ladder, visit <u>www.neb.com/</u> <u>DNAmarkersandladders</u>





Why is Polymerase Fidelity Important?

What is fidelity?

The fidelity of a DNA polymerase refers to its ability to accurately replicate a template. A critical aspect of this is the ability of the DNA polymerase to read a template strand, select the appropriate nucleoside triphosphate and insert the correct nucleotide at the 3′ primer terminus, such that canonical Watson-Crick base pairing is maintained. The rate of misincorporation is known as the polymerase's error rate. In addition to effective discrimination for correct over incorrect nucleotide incorporation, some DNA polymerases possess a 3′→5′ exonuclease activity. This activity, also termed proofreading, is used to excise incorrectly incorporated mononucleotides that are then replaced with the correct nucleotide. High-fidelity PCR utilizes DNA polymerases that couple low misincorporation rates with proofreading activity to give faithful replication of the DNA target of interest.

For what applications is fidelity important?

Fidelity is important for applications in which the DNA sequence must be correct after amplification, including:

- Cloning/subcloning from in vitro amplified material (PCR, WGA, etc) for protein expression or gene studies
- · SNP analysis by cloning and sequencing
- RNA analysis by RT-PCR
- Applications that involve sequencing of in vitro amplified material

Fidelity is less important if the PCR amplified product is directly sequenced by Sanger sequencing (without an intervening cloning step), where the signal is an average of the input amplicons. Fidelity is also less important for diagnostic applications in which sequencing is not required after amplification, and the read-out is the presence or absence of a product. It is more important for next generation and single molecule sequencing techniques.

How does a high-fidelity polymerase ensure that the correct base is inserted?

High-fidelity DNA polymerases have several checkpoints to protect against making and propagating mistakes while copying DNA.

- High-fidelity polymerases have a significant binding preference for the correct versus the incorrect nucleotide triphosphate during polymerization.
- If an incorrect nucleotide does bind in the polymerase active site, incorporation is slowed due to the sub-optimal architecture of the active site complex. This time increases the opportunity for the incorrect nucleotide to dissociate before incorporation, thereby allowing the process to start again (and for a correct nucleotide triphosphate to bind) (1,2).
- If an incorrect nucleotide is inserted, proofreading DNA polymerases have an extra line of defense. They can "sense" the perturbation caused by the mispaired bases and move the 3′ end of the growing DNA chain into a proofreading 3′→5′ exonuclease domain. There, the incorrect nucleotide is removed by the 3′→5′ exonuclease activity before the chain is moved back into the polymerase domain, where polymerization can continue with the correct nucleotide.

TOOLS & RESOURCES

Visit www.neb.com/tools-and-resources/tutorials to find the latest PCR videos from NEB Scientists, including:

- · Choosing the right polymerase for your PCR
- · How to amplify GC-rich DNA
- · Why choose Q5 High-Fidelity DNA Polymerase
- Important tips for Q5 High-Fidelity DNA Polymerase
- Tips for amplifying large amplicons
- · Amplification of GC-rich regions
- · Tips for setting up PCR
- Types of PCR
- Why is Tm important?



FEATURED NEB PUBLICATION

Learn how PacBio sequencing was used to better understand sources of error introduced by PCR

Potapov, V. and Ong, J.L. (2017) PLOS One, 12(1): e 0169774



References:

- Johnson, K. A. (2010) Biochim. et. Biophys. Acta, 1804, 1041–1048.
- 2. Joyce, C. M. and Bencovic, S. (2004) *Biochemistry*, 43, 14317–14324.



General Guidelines for PCR Optimization

New England Biolabs offers a diverse group of DNA Polymerases for PCR-based applications. Specific recommendations for PCR optimization can be found in the product literature or on the individual product webpages. However, these general guidelines will help to ensure success using New England Biolabs' PCR enzymes.

Setup Guidelines

DNA Template

- Use high quality, purified DNA templates whenever possible. Please refer to specific product information for amplification from unpurified DNA (e.g., colony PCR or direct PCR).
- For low complexity templates (e.g., plasmid, lambda, BAC DNA), use 1 pg-10 ng of DNA per 50 μl reaction
- For higher complexity templates
 (e.g., genomic DNA), use 1 ng–1 μg of DNA
 per 50 μl reaction
- Higher DNA concentrations tend to decrease amplicon specificity, particularly for high numbers of cycles

Primers

- Primers should typically be 20–30 nucleotides in length, with 40–60% GC Content
- Primer Tm values should be determined with NEB's Tm Calculator (TmCalculator.neb.com)
- Primer pairs should have Tm values that are within 5°C
- Avoid secondary structure (e.g., hairpins) within each primer and potential dimerization between the primers
- Higher than recommended primer concentrations may decrease specificity
- When engineering restriction sites onto the end of primers, 6 nucleotides should be added 5' to the site
- Annealing temperatures should be determined according to specific enzyme recommendations. Please note that Q5 and Phusion* annealing temperature recommendations are unique.

- Final concentration of each primer should be 0.05–1 µM in the reaction. Please refer to the more detailed recommendations for each specific enzyme.
- When amplifying products > 20 kb in size, primers should be ≥ 24 nucleotides in length with a GC content above 50% and matched Tm values above 60°C
- To help eliminate primer degradation and subsequent non-specific product formation, use a hot-start enzyme (e.g., One *Taq* Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase)

Magnesium Concentration

- Optimal Mg²⁺ concentration is usually 1.5–2.0 mM for most PCR polymerases
- Most PCR buffers provided by NEB already contain sufficient levels of Mg²⁺ at 1X concentrations.
- NEB offers a variety of Mg-free reaction buffers to which supplemental Mg²⁺ can be added for applications that require complete control over Mg²⁺ concentration
- Further optimization of Mg²⁺ concentration can be done in 0.2–1 mM increments, if necessary. For some specific applications, the enzyme may require as much as 6 mM Mg²⁺ in the reaction.
- Excess Mg²⁺ may lead to spurious amplification; Insufficient Mg²⁺ concentrations may cause reaction failure

Deoxynucleotides

- Ideal dNTP concentration is typically 200 μM of each, however, some enzymes may require as much as 400 μM each. Please refer to specific product literature for more detailed recommendations.
- Excess dNTPs can chelate Mg²⁺ and inhibit the polymerase
- Lower dNTP concentration can increase fidelity, however, yield is often reduced
- The presence of uracil in the primer, template, or deoxynucleotide mix will cause reaction failure when using archaeal PCR polymerases. Use Q5U, One *Taq*, or *Taq* DNA polymerases for these applications.

Enzyme Concentration

- Optimal enzyme concentration in the reaction is specific to each polymerase.
 Please see the product literature for specific recommendations.
- In general, excess enzyme can lead to amplification failure, particularly when amplifying longer fragments

Starting Reactions

- Unless using a hot start enzyme (e.g., One *Taq* Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase), assemble all reaction components on ice
- Add the polymerase last, whenever possible
- Transfer reactions to a thermocycler that has been pre-heated to the denaturation temperature. Please note that pre-heating the thermocycler is not necessary when using a hot start enzyme (e.g., One *Taq* Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase).



Cycling Guidelines

Denaturation

- Optimal denaturation temperature ranges from 94°–98°C and is specific to the polymerase in the reaction.
 Please refer to product information for recommended conditions.
- Avoid longer or higher temperature incubations unless required due to high GC content of the template
- For most PCR polymerases, denaturation of 5–30 seconds is recommended during cycling
- NEB's aptamer-based hot start enzymes do not require additional denaturation steps to activate the enzymes

Annealing

- Primer Tm values should be determined using the NEB Tm Calculator (<u>TmCalculator.neb.com</u>)
- For PCR polymerases other than Q5 High-Fidelity DNA Polymerase or Phusion
 High-Fidelity DNA Polymerase*, annealing
 temperatures are usually set at 2°-5°C below
 the lowest Tm of the primer pair
- When using Q5 High-Fidelity DNA
 Polymerase or Phusion High-Fidelity DNA
 Polymerase*, annealing temperatures should
 be set at 0°-3°C above the lowest Tm of
 the primer pair. Please refer to the product
 literature for detailed recommendations.
- Non-specific product formation can often be avoided by optimizing the annealing temperature or by switching to a hot start enzyme (e.g., One *Taq* Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase)

- Annealing temperatures can be optimized by doing a temperature gradient PCR, starting at 5°C below the lowest Tm of the primer pair
- Ideally, primer Tm values should be less than the extension temperature. However, if Tm values are calculated to be greater than the extension temperature, a two-step PCR program (combining annealing and extension into one step) can be employed.

Extension

- Extension temperature recommendations range from 65°–72°C and are specific to each PCR polymerase. Please refer to the product literature for specific recommendations.
- Extension rates are specific to each PCR polymerase. In general, extension rates range from 15–60 seconds per kb. Please refer to the recommendations for each specific product.
- Longer than recommended extension times can result in higher error rates, spurious banding patterns and/or reduction of amplicon yields

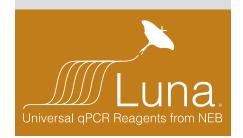


For more information on polymerase properties and usage, visit www.neb.com.

qPCR & RT-qPCR GUIDELINES

Are you doing qPCR or RT-qPCR?

Visit www.neb.com/qPCRguidelines or www.neb.com/RTqPCRguidelines for optimization tips when using Luna qPCR & RT-qPCR products.



^{*}Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific. Phusion® is a registered trademark and property of Thermo Fisher Scientific.



PCR Polymerases

| PRODUCT | NEB # | SIZE |
|--|--------------|--|
| Deep Vent DNA Polymerase | M0258S/L | 200/1,000 units |
| Deep Vent (exo ⁻) DNA Polymerase | M0259S/L | 200/1,000 units |
| EpiMark Hot Start <i>Taq</i> DNA Polymerase | M0490S/L | 100/500 reactions |
| Hemo KlenTaq DNA Polymerase | M0332S/L | 200/1,000 reactions (25 µl reaction vol) |
| Hot Start <i>Taq</i> 2X Master Mix | M0496S/L | 100/500 reactions (50 µl reaction vol) |
| Hot Start <i>Taq</i> DNA Polymerase | M0495S/L | 200/1,000 units |
| LongAmp <i>Taq</i> 2X Master Mix | M0287S/L | 100/500 reactions (50 µl reaction vol) |
| LongAmp <i>Taq</i> DNA Polymerase | M0323S/L | 500/2,500 units |
| LongAmp <i>Taq</i> PCR Kit | E5200S | 100 reactions (50 µl reaction vol) |
| LongAmp Hot Start <i>Taq</i> DNA Polymerase | M0534S/L | 500/2,500 units |
| LongAmp Hot Start <i>Taq</i> 2X Master Mix | M0533S/L | 100/500 reactions (50 µl reaction vol) |
| Multiplex PCR 5X Master Mix | M0284S | 100 reactions (50 µl reaction vol) |
| NEBNext High-Fidelity 2X PCR Master Mix | M0541S/L | 50/250 reactions |
| NEBNext Ultra II Q5 Master Mix | M0544S/L | 50/250 reactions |
| One <i>Taq</i> DNA Polymerase | M0480S/L/X | 200/1,000/5,000 units |
| One <i>Taq</i> 2X Master Mix with Standard Buffer | M0482S/L | 100/500 reactions (50 µl reaction vol) |
| One <i>Taq</i> Quick-Load 2X Master Mix with Standard Buffer | M0486S/L | 100/500 reactions (50 µl reaction vol) |
| One <i>Tag</i> Hot Start DNA Polymerase | M0481S/L/X | 200/1,000/5,000 units |
| One <i>Taq</i> Hot Start 2X Master Mix with Standard Buffer | M0484S/L | 100/500 reactions (50 µl reaction vol) |
| One Tag Hot Start 2X Master Mix with GC Buffer | M0485S/L | 100/500 reactions (50 µl reaction vol) |
| One <i>Tag</i> Hot Start Quick-Load 2X Master Mix with Standard Buffer | M0488S/L | 100/500 reactions (50 µl reaction vol) |
| One Tag Hot Start Quick-Load 2X Master Mix with GC Buffer | M0489S/L | 100/500 reactions (50 µl reaction vol) |
| · | | , , , |
| One Tag Quick-Load DNA Polymerase | M0509S/L/X | 100/500/2,500 units |
| Phusion High-Fidelity DNA Polymerase | M0530S/L | 100/500 units |
| Phusion High-Fidelity PCR Kit | E0553S/L | 50/200 reactions (50 μl reaction vol) |
| Phusion High-Fidelity PCR Master Mix with HF Buffer | M0531S/L | 100/500 reactions (50 µl reaction vol) |
| Phusion High-Fidelity PCR Master Mix with GC Buffer | M0532S/L | 100/500 reactions (50 µl reaction vol) |
| Phusion Hot Start Flex DNA Polymerase | M0535S/L | 100/500 units |
| Phusion Hot Start Flex 2X Master Mix | M0536S/L | 100/500 reactions (50 µl reaction vol) |
| Q5 High-Fidelity DNA Polymerase | M0491S/L | 100/500 units |
| Q5 Hot Start High-Fidelity DNA Polymerase | M0493S/L | 100/500 units |
| Q5 High-Fidelity 2X Master Mix | M0492S/L | 100/500 reactions (50 μl reaction vol) |
| Q5 Hot Start High-Fidelity 2X Master Mix | M0494S/L/X | 100/500/500 reactions (50 μl reaction vol) |
| Q5 Blood Direct 2X Master Mix | M0500S/L | 100/500 reactions (20 µl reaction vol) |
| Q5U Hot Start High-Fidelity DNA Polymerase | M0515S/L | 100/500 units |
| NEBNext Q5U Master Mix | M0597S/L | 50/250 reactions |
| Quick-Load <i>Taq</i> 2X Master Mix | M0271L | 500 reactions (50 µl reaction vol) |
| Taq 2X Master Mix | M0270L | 500 reactions (50 μl reaction vol) |
| Tag 5X Master Mix | M0285L | 500 reactions (50 μl reaction vol) |
| Tag DNA Polymerase with Standard Tag Buffer | M0273S/L/X/E | 400/2,000/4,000/20,000 units |
| Taq DNA Polymerase with Standard Taq (Mg-free) Buffer | M0320S/L | 400/2,000 units |
| Taq DNA Polymerase with ThermoPol Buffer | M0267S/L/X/E | 400/2,000/4,000/20,000 units |
| Tag PCR Kit | E5000S | 200 reactions (50 μl reaction vol) |
| Vent DNA Polymerase | M0254S/L | 200/1,000 units |
| Vent (exo⁻) DNA Polymerase | M0257S/L | 200/1,000 units |

Repair

| PRODUCT | NEB # | SIZE |
|-----------------------------|----------|------------------|
| PreCR Repair Mix | M0309S/L | 30/150 reactions |
| NEBNext FFPE DNA Repair Mix | M6630S/L | 24/96 reactions |

 $Visit \ \underline{\textbf{LUNAqPCR.com}} \ for \ ordering \ and \ sample \ information \ for \ Luna \ qPCR \ and \ RT-qPCR \ products.$

Companion Products

| PRODUCT | NEB # | SIZE |
|---|----------|------------------------------------|
| 1 kb DNA Ladder | N3232S/L | 200/1,000 gel lanes |
| Quick-Load 1 kb Extend DNA Ladder | N3239S | 125 gel lanes |
| 100 bp DNA Ladder | N3231S/L | 100/500 gel lanes |
| 1 kb Plus DNA Ladder | N3200S/L | 100-200/500-1,000 gel lanes |
| 50 bp DNA Ladder | N3236S/L | 100-200/500-1,000 gel lanes |
| Low Molecular Weight DNA Ladder | N3233S/L | 100/500 gel lanes |
| Fast DNA Ladder | N3238S | 50 gel lanes |
| PCR Marker | N3234S/L | 100/500 gel lanes |
| Quick-Load Purple 1 kb DNA Ladder | N0552S/L | 125/375 gel lanes |
| Quick-Load Purple 50 bp DNA Ladder | N0556S | 250 gel lanes |
| Quick-Load Purple 100 bp DNA Ladder | N0551S/L | 125/375 gel lanes |
| Quick-Load Purple 1 kb Plus DNA Ladder | N0550S/L | 125-250/375-750 gel lanes |
| Quick-Load Purple Low Molecular Weight DNA Ladder | N0557S | 125 gel lanes |
| Deoxynucleotide Solution Set | N0446S | 25 μmol of each |
| Deoxynucleotide Solution Mix | N0447S/L | 8 μmol of each/40 μmol of each |
| dATP Solution | N0440S | 25 μmol |
| Acyclonucleotide Set | N0460S | 0.5 µmol of each |
| 7-deaza-dGTP | N0445S/L | 0.15 µmol of each/0.3 µmol of each |
| Ribonucleotide Solution Set | N0450S/L | 10 μmol of each/50 μmol of each |
| Ribonucleotide Solution Mix | N0466S/L | 8 µmol of each/40 µmol of each |
| Monarch® Plasmid Miniprep Kit | T1010S/L | 50/250 preps |
| Monarch DNA Gel Extraction Kit | T1020S/L | 50/250 preps |
| Monarch PCR & DNA Cleanup Kit (5 µg) | T1030S/L | 50/250 preps |
| Exo-CIP™ Rapid PCR Cleanup Kit | E1050S/L | 100/400 reactions |

Australia & New Zealand

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