



Now includes  
Q5<sup>®</sup> Blood Direct 2X Master Mix

# PCR Reagents

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POLYMERASES, NUCLEOTIDES,  
& DNA LADDERS



NEW ENGLAND

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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 [nebpcr.com](http://nebpcr.com).

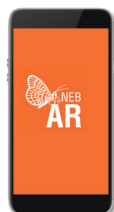
## Featured Products Include:

- **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
- **OneTaq 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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Find an overview of PCR.



\*see back cover for details

## TABLE OF CONTENTS

- 3 PCR Polymerase Selection Chart
- 4–8 High-Fidelity PCR
  - 4 Q5® and Q5U® products
  - 8 Phusion® products
- 6–9 Routine PCR
  - 6 OneTaq® products
  - 9 Taq products
- 10–12 Specialty PCR
  - 10 Q5® Blood Direct 2X Master Mix
  - 10 EpiMark® Hot Start Taq
  - 11 LongAmp® Taq
  - 11 Multiplex PCR 5X Master Mix
  - 12 PreCR® Repair Mix
- 12 PCR Cleanup
  - 12 Exo-CIP™ Rapid PCR Cleanup Kit
  - 12 Monarch® PCR & DNA Cleanup Kit
- 13–14 Complementary Products
  - 13 Nucleotide Solutions
  - 14 DNA Analysis
- 15 Why is Polymerase Fidelity Important?
- 16–17 General Guidelines
- 18–19 Ordering Information

## Related Resources at [neb.com](http://neb.com):

- Visit [LUNAqPCR.com](http://LUNAqPCR.com) to learn more about the Luna portfolio of products for qPCR & RT-qPCR
- Visit [www.neb.com/isoamp](http://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 [NEBrna.com](http://NEBrna.com) to find products supporting RNA workflows
- Visit [www.neb.com/supporting-molecular-diagnostics](http://www.neb.com/supporting-molecular-diagnostics) to find amplification-based products for molecular diagnostic applications



# PCR Polymerase Selection Chart

When choosing a polymerase for PCR, we recommend starting with OneTaq® or Q5® 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

PROPERTIES	STANDARD PCR		HIGH-FIDELITY PCR			SPECIALTY PCR				
	OneTaq® / OneTaq Hot Start	Taq / Hot Start Taq	HIGHEST FIDELITY		MODERATE FIDELITY	LONG AMPLICONS	dU TOLERANCE		BLOOD DIRECT PCR	
			Q5®/Q5 Hot Start	Phusion®(4) / Phusion Flex	Vent® / Deep Vent™	LongAmp® / LongAmp Hot Start Taq	Q5U®	Epimark® Hot Start Taq	Q5 Blood	Hemo KlenTaq®
<b>Fidelity vs. Taq</b>	2X	1X	~280X <sup>(2)</sup>	> 39X	5–6X	2X	ND	1X	ND	ND
<b>Amplicon Size</b>	< 6 kb	≤ 5 kb	≤ 20 kb	≤ 20 kb	≤ 6 kb	≤ 30 kb	app-specific	≤ 1 kb	≤ 7.5 kb	≤ 2 kb
<b>Extension Time</b>	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
<b>Resulting Ends</b>	3' A/Blunt	3' A	Blunt	Blunt	Blunt	3' A/Blunt	Blunt	3' A	Blunt	3' A
<b>3'→5' exo</b>	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	No
<b>5'→3' exo</b>	Yes	Yes	No	No	No	Yes	No	Yes	No	No
<b>Units/50 µl Reaction</b>	1.25	1.25	1.0	1.0	0.5–1.0	5.0	1.0	1.25	N/A	N/A
<b>Annealing Temperature</b>	Tm-5	Tm-5	Tm-3	Tm-3	Tm-5	Tm-5	Tm-3	Tm-5	Tm-3	Tm-5
<b>APPLICATIONS</b>										
<b>Routine PCR</b>	★	●	●	●	●	●				
<b>Colony PCR</b>	★	●								
<b>Enhanced Fidelity</b>	●		★	●	●	●	●		●	
<b>High Fidelity</b>			★	●			●		●	
<b>High Yield</b>	★	●	★	●					●	
<b>Fast</b>			★	●					●	
<b>Long Amplicon</b>			★	●		★				
<b>GC-rich Targets</b>	★		★		●	●			●	
<b>AT-rich Targets</b>	★	●	★	●		●	★	●		
<b>High Throughput</b>	●	●	●	●			★	●		
<b>Multiplex PCR</b>	●	★ <sup>(1)</sup>	●	●					●	
<b>Extraction-free PCR</b>									★	●
<b>DNA Labeling</b>		★								
<b>Site-directed Mutagenesis</b>			★	●						
<b>Carryover Prevention</b>							★	●		
<b>USER® Cloning</b>							★	●		
<b>NGS APPLICATIONS</b>										
<b>NGS Library Amplification</b>			★ <sup>(3)</sup>	●			★ <sup>(6)</sup>	★ <sup>(5)</sup>		
<b>FORMATS</b>										
<b>Hot Start Available</b>	●	●	●	●		●	●	●	●	
<b>Kit</b>		●	●	●		●				
<b>Master Mix Available</b>	●	●	●	●		●			●	
<b>Direct Gel Loading</b>	●	●								

- (1) Use Multiplex PCR 5X Master Mix.
- (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.
- (5) Use NEBNext Enzymatic Methyl-seq Kit (EM-seq™) for Illumina.
- (6) Use NEBNext Q5U Master Mix.

Why choose Q5 for your PCR?





# Q5® High-Fidelity DNA Polymerase

## Q5 Hot Start High-Fidelity DNA Polymerase

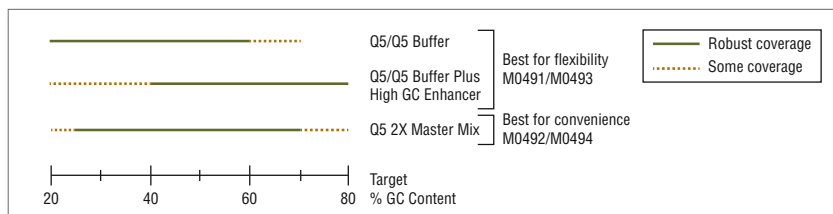
Q5 High-Fidelity DNA Polymerase sets a new standard for both fidelity and performance. With the highest fidelity amplification available (~280X higher than *Taq* and > 5X higher than Thermo Scientific® Phusion®), Q5 DNA Polymerase results in ultra-low error rates. Q5 DNA Polymerase is composed of a novel 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](http://NEBNext.com) for details.

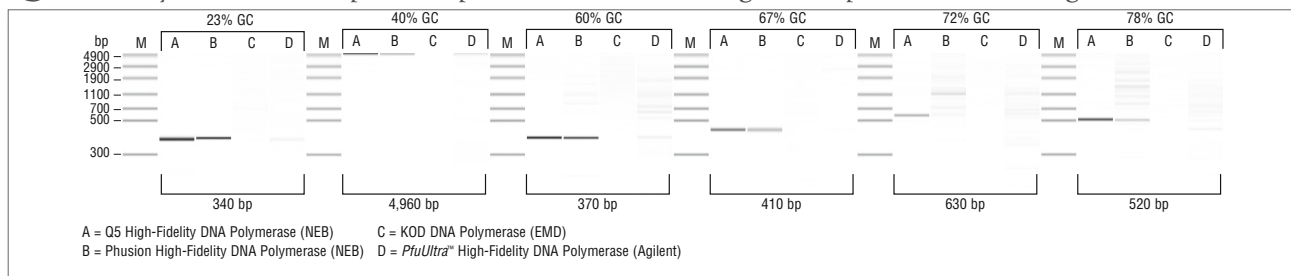
Q5 High-Fidelity DNA Polymerase	M0491S/L
Q5 High-Fidelity 2X Master Mix	M0492S/L
Q5 Hot Start High-Fidelity DNA Polymerase	M0493S/L
Q5 Hot Start High-Fidelity 2X Master Mix	M0494S/L
<b>NEW</b> Q5 Blood Direct 2X Master Mix	M0500S/L
Q5 High-Fidelity PCR Kit	E0555S/L
NEBNext® High-Fidelity 2X PCR Master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

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.

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.

### Fidelity at its finest.



Extension Rate	6 kb/min
Amplicon Size	≤ 20 kb
Fidelity	~280X <i>Taq</i>
Units/50 µl rxn.	1 unit
Resulting Ends	Blunt
3'→5' Exonuclease Activity	Yes
5'→3' Exonuclease Activity	No
Supplied Buffer	Q5 Rxn Buffer
Supplied Enhancer	Q5 High GC Enhancer
Compatible w/Other Buffers	with Reduced Activity Profile

#### Product Formats

Hot Start Available	Yes
- Activation Required	No
Master Mix Available	Yes
PCR Kit Available	Yes
NGS Version Available	Yes

#### Applications

High-Fidelity PCR	Yes
Difficult PCR	Yes
High GC PCR	Yes
T/A, U/A Cloning	No
Colony PCR	No
Blunt Cloning	Yes



Learn how Q5 can be used in multiplex PCR in our application note at [Q5PCR.com](http://Q5PCR.com)





## Comparison of high-fidelity polymerases

PRODUCT NAME (SUPPLIER)	POLYMERASE FIDELITY (Reported by supplier)	MAXIMUM AMPLICON LENGTH <sup>5</sup>	EXTENSION TIME <sup>5</sup> (For simple templates <sup>4</sup> )	EXTENSION TIME <sup>5</sup> (For complex templates <sup>4</sup> )
Q5 High-Fidelity DNA Polymerase (NEB)	~280X <i>Taq</i> <sup>1</sup>	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> <sup>1</sup>	20 kb simple; 10 kb complex	15 s/kb	30 s/kb
AccuPrime™ <i>Pfx</i> (Life)	26X <i>Taq</i> <sup>2</sup>	12 kb <sup>3</sup>	60 s/kb <sup>3</sup>	
<i>PfuUltra</i> ™ II Fusion HS (Agilent)	20X <i>Taq</i> <sup>2</sup>	19 kb <sup>3</sup>	15 s/kb (< 10 kb <sup>3</sup> ) 30 s/kb (> 10 kb <sup>3</sup> )	
<i>PfuUltra</i> High-Fidelity DNA Polymerase (Agilent)	19X <i>Taq</i> <sup>2</sup>	17 kb simple; 6 kb complex	60 s/kb (< 10 kb) 120 s/kb (> 10 kb)	60 s/kb (< 6 kb) 120 s/kb (> 6 kb)
KOD DNA Polymerase (EMD)	12X <i>Taq</i> <sup>1</sup>	6 kb simple; 2 kb complex	10–20 s/kb	30–60 s/kb
Platinum® <i>Taq</i> HiFi (Life)	6X <i>Taq</i> <sup>2</sup>	20 kb <sup>3</sup>	60 s/kb <sup>3</sup>	

<sup>1</sup> 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.).

<sup>2</sup> 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.

<sup>3</sup> Template not specified.

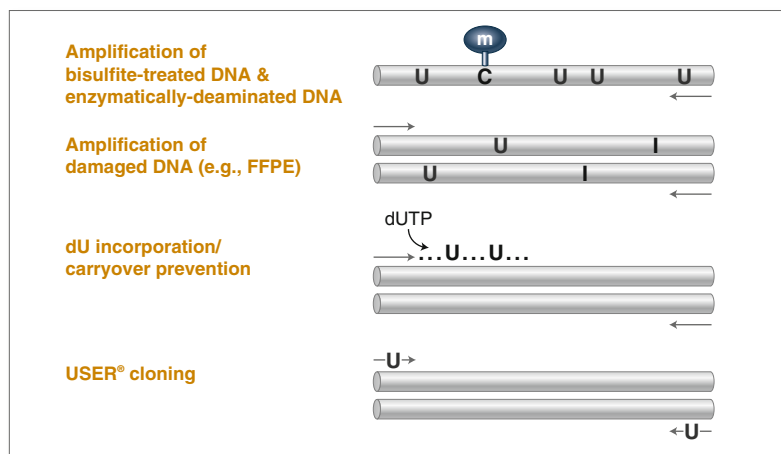
<sup>4</sup> Simple templates include plasmid, viral and *E. coli* genomic DNA. Complex templates include plant, human and other mammalian genomic DNA.

<sup>5</sup> Values provided by individual manufacturers.

Q5U<sup>®</sup> 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.



# OneTaq<sup>®</sup> DNA Polymerase OneTaq Hot Start DNA Polymerase

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

*OneTaq* 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, *OneTaq* Standard Reaction Buffer provides robust amplification. For GC-rich amplicons, the *OneTaq* GC Reaction Buffer can improve both performance and yield. For particularly high GC (> 65%) or difficult amplicons, the *OneTaq* High GC Enhancer can be added to reactions containing *OneTaq* GC Buffer. These formulations ensure maximum performance for routine, AT- or GC-rich amplicons.

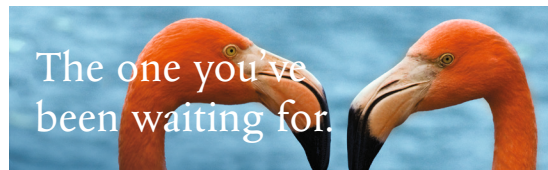
## Master Mix Formulations

In addition to standalone enzymes, both *OneTaq* and *OneTaq* Hot Start DNA Polymerases are available in master mix and Quick-Load<sup>®</sup> master mix formats. Master mix formulations include dNTPs, MgCl<sub>2</sub> 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.

<i>OneTaq</i> DNA Polymerase .....	M0480S/L/X
<i>OneTaq</i> 2X Master Mix with Standard Buffer .....	M0482S/L
<i>OneTaq</i> Quick-Load 2X Master Mix with Standard Buffer .....	M0486S/L
<i>OneTaq</i> Quick-Load DNA Polymerase .....	M0509S/L

## OneTaq Buffer Recommendations

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



Extension Rate .....	1 kb/min
Amplicon Size .....	≤ 6 kb
Fidelity .....	2X <i>Taq</i>
Units/50 µl rxn .....	1.25 units
Resulting Ends .....	3' A/Blunt
3'→5' Exonuclease Activity .....	Yes
5'→3' Exonuclease Activity .....	Yes
Supplied Buffer .....	<i>OneTaq</i> Std Rxn Buffer, <i>OneTaq</i> GC Rxn Buffer
Supplied Enhancer .....	<i>OneTaq</i> High GC Enhancer
Compatible w/Other Buffers .....	with Reduced Activity Profile

### Product Formats

Hot Start Available .....	Yes
- Activation Required .....	No
Master Mix Available .....	Yes
Direct Gel-loading Available .....	Yes
PCR Kit Available .....	No

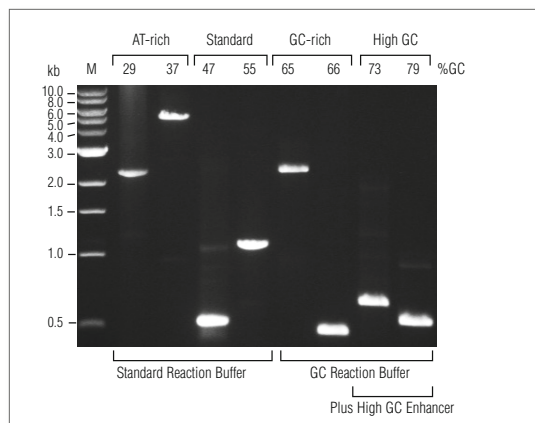
### Applications

Routine PCR .....	Yes
SNP Detection .....	Yes
T/A, U/A Cloning .....	Yes
Colony PCR .....	Yes



Visit [www.neb.com/OneTaq](http://www.neb.com/OneTaq) for more information.

Achieve robust amplification for routine, AT- and GC-rich templates with *OneTaq*



Amplification of a selection of sequences with varying AT and GC content from human and *C. elegans* genomic DNA using *OneTaq* DNA Polymerase. GC content is indicated above gel. Marker M is the 1 kb DNA Ladder (NEB #N3232).





## OneTaq Hot Start DNA Polymerase allows room temperature reaction setup with no separate activation step



To learn how OneTaq can be used in colony PCR, download the application note at [www.neb.com/OneTaq](http://www.neb.com/OneTaq)

In contrast to chemically-modified or antibody-based hot start polymerases, NEB's OneTaq 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. OneTaq 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.

- OneTaq Hot Start DNA Polymerase ..... M0481S/L/X
- OneTaq Hot Start 2X Master Mix with Standard Buffer ..... M0484S/L
- OneTaq Hot Start 2X Master Mix with GC Buffer ..... M0485S/L
- OneTaq Hot Start Quick-Load 2X Master Mix with Standard Buffer ..... M0488S/L
- OneTaq Hot Start Quick-Load 2X Master Mix with GC Buffer ..... M0489S/L

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

Polymerase	Additives	GC%					
		55	65	66	67	78	79
<b>OneTaq® DNA Polymerase (NEB)</b>	None*	●	●	●	●	●	●
<b>OneTaq HotStart DNA Polymerase (NEB)</b>	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 Polymerase 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

% Purity

0 100

\* OneTaq products are supplied with OneTaq Standard Reaction Buffer and OneTaq GC Reaction Buffer. The GC reaction buffer was used to amplify the targets shown in the table. For other products, amplification reactions were conducted both with and without GC enhancers (if provided).

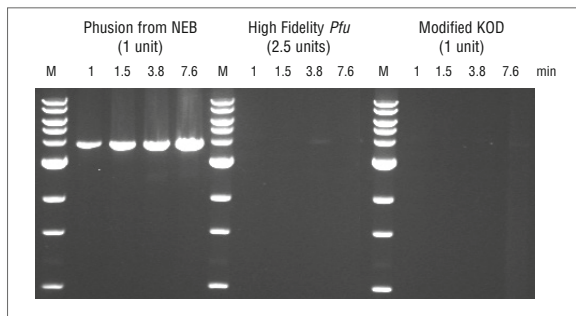


# Phusion® High-Fidelity DNA Polymerase

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

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



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).

## POLYMERASE DETAILS

Extension Rate.....	4 kb/min
Amplicon Size.....	≤ 20 kb
Fidelity .....	39X <i>Taq</i>
Units/50 µl rxn.....	1 units
Resulting Ends.....	Blunt
3'→5' Exonuclease Activity.....	Yes
5'→3' Exonuclease Activity.....	No
Supplied Buffer.....	5X Phusion HF Buffer, 5X Phusion GC Buffer
Supplied Enhancer.....	100% DMSO
Compatible w/Other Buffers.....	No

## Product Formats

Hot Start Available.....	Yes
- Activation Required.....	No
Master Mix Available.....	Yes
PCR Kit Available.....	Yes

## Applications

High-Fidelity PCR.....	Yes
T/A, U/A Cloning.....	No
Colony PCR.....	No
Blunt Cloning.....	Yes



High-fidelity polymerases benefit from a  $T_m + 3$  annealing temperature. Use the NEB Tm Calculator to ensure successful PCR at [TmCalculator.neb.com](http://TmCalculator.neb.com).

## 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.





## 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.

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

Looking for a hot start *Taq* for use in molecular diagnostics?  
Contact us at [custom@neb.com](mailto: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 compatible with existing assay systems	<i>Taq</i> with Standard <i>Taq</i> Buffer	M0273S/L/X
	<i>Taq</i> with Standard <i>Taq</i> (Mg-free) Buffer	M0320S/L
ThermoPol Buffer: Designed to optimize yields and specificity	<i>Taq</i> with ThermoPol Buffer	M0267S/L/X/E

### POLYMERASE 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 Buffer.....	Standard <i>Taq</i> Rxn Buffer, or ThermoPol Rxn Buffer
Compatible w/Other <i>Taq</i> Buffers.....	Yes

### Product Formats

Hot Start Available.....	Yes
- Activation Required.....	No
Master Mix Available.....	Yes
Direct Gel-loading Available.....	Yes
PCR Kit Available.....	Yes

### Applications

Routine PCR.....	Yes
SNP Detection.....	Yes
T/A, U/A Cloning.....	Yes
Colony PCR.....	Yes

## Luna® Universal qPCR & RT-qPCR Products

Rapid, sensitive and precise dye- or probe-based detection and quantitation of DNA and RNA

- Visible tracking dye reduces pipetting errors
- Novel thermostable reverse transcriptase improves performance
- Compatible with high-throughput workflows
- Increase sensitivity of your RT-qPCR, as 4X concentration allows for more sample input (#M3019, #M3029)

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stay GENUINE



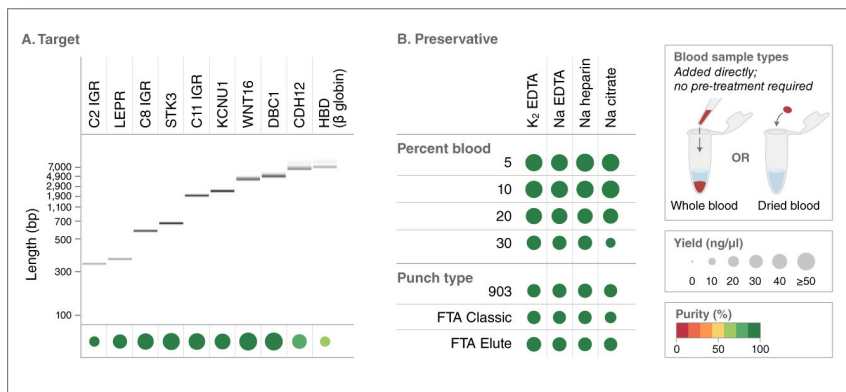


## Q5® Blood Direct 2X Master Mix

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.

EpiMark Hot Start Taq DNA Polymerase ..... M0490S/L

### 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



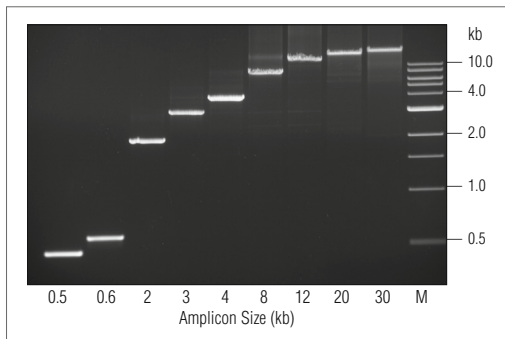


## LongAmp® *Taq* enables extension of longer amplicons

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 <i>Taq</i> DNA Polymerase .....	M0323S/L
LongAmp <i>Taq</i> PCR Kit .....	E5200S
LongAmp <i>Taq</i> 2X Master Mix .....	M0287S/L
LongAmp Hot Start <i>Taq</i> DNA Polymerase .....	M0534S/L
LongAmp Hot Start <i>Taq</i> 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

Extension Rate .....	1.2 kb/min
Amplicon Size .....	≤ 30 kb
Fidelity .....	2X <i>Taq</i>
Units/50 µl rxn .....	5 units
Resulting Ends .....	3' A/Blunt
3' → 5' Exonuclease Activity .....	Yes
5' → 3' Exonuclease Activity .....	Yes
Supplied Buffer .....	LongAmp or <i>Taq</i> Rxn Buffer
Compatible w/Other <i>Taq</i> Buffers .....	with Reduced Activity Profile

### Product Formats

Hot Start Available .....	Yes
- Activation Required .....	No
Master Mix Available .....	Yes
Direct Gel-loading Available .....	Yes
PCR Kit Available .....	Yes

### Applications

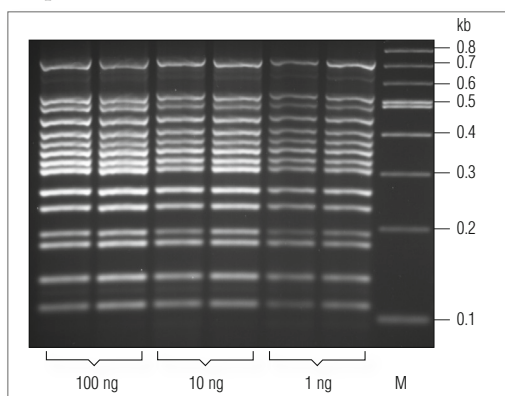
Long Amplicons .....	Yes
Routine PCR .....	Yes
T/A, U/A Cloning .....	Yes
Colony PCR .....	Yes

## 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
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### 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<sup>®</sup> 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 ..... M0309S/L

For repair of DNA prior to next generation sequencing library preparation, we recommend the NEBNext FFPE DNA Repair Mix (NEB #M6630). Visit [NEBNext.com](http://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<sup>™</sup> 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 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 ..... E1050S/L

### ADVANTAGES

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

## Monarch<sup>®</sup> PCR & DNA Cleanup Kit (5 µg)

The Monarch PCR & DNA Cleanup Kit rapidly and reliably purifies up to 5 µg 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 µl. 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.

Monarch PCR & DNA Cleanup Kit ..... T1030S/L

### 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).	N0446S
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.	N0447S/L
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. <i>*licensed from Roche Diagnostics GmbH</i>	N0445S/L
Acyclonucleotide Set	Acyclonucleotide Set contains four separate tubes of acyNTPs (acyATP, acyCTP, acyGTP and acyTTP).	N0460S
dATP Solution	Contains 0.25 ml of 100 mM ultrapure dATP.	N0440S
Ribonucleotide Solution Set	Ribonucleotide Solution Set consists of four separate 100 mM solutions of ATP, GTP, CTP and UTP.	N0450S/L
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

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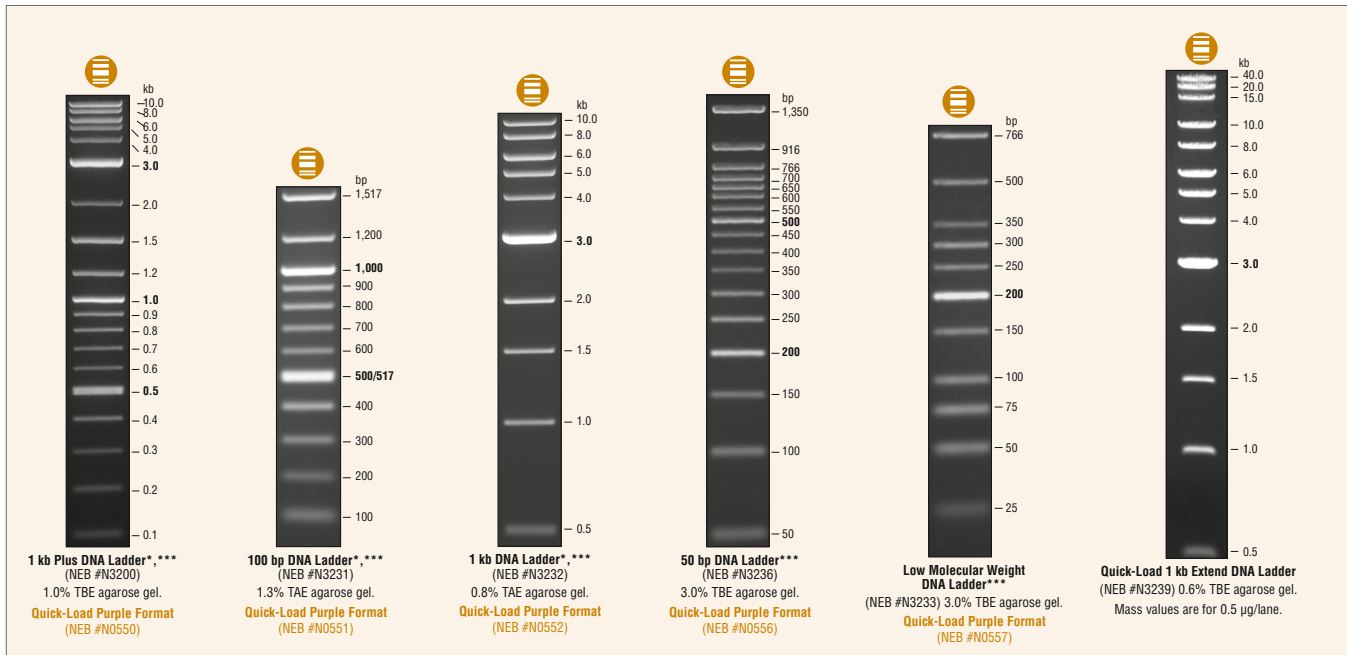




# DNA Analysis

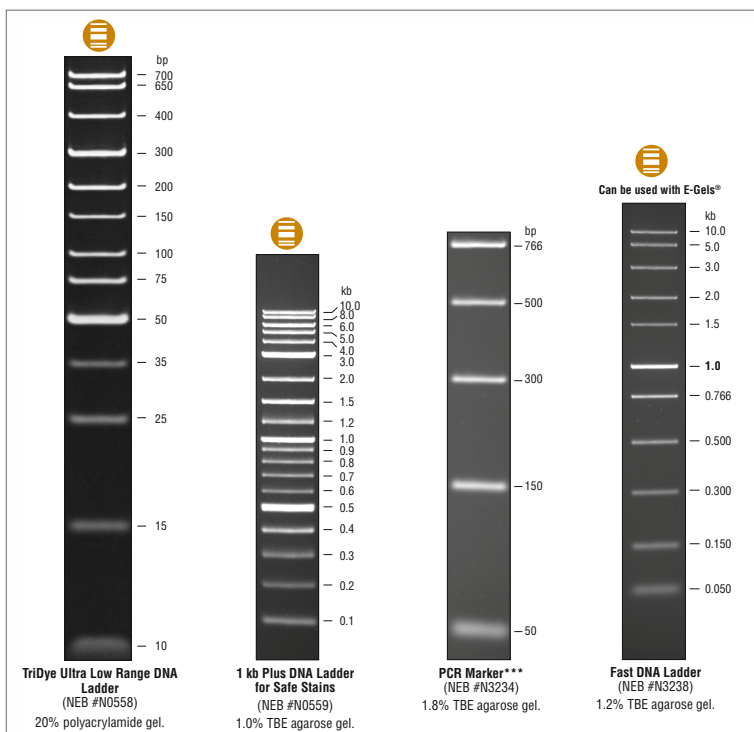
Agarose-gel electrophoresis is the standard method used for separation, identification and purification of DNA fragments. DNA is visualized on a gel after soaking or pre-casting the gel with a visualization dye, such as Ethidium Bromide, which is a DNA intercalating agent that fluoresces under UV illumination. 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      Ready-to-Load      \*\*\* 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 [www.neb.com/DNAmarkersandladders](http://www.neb.com/DNAmarkersandladders)

**Ready-to-load DNA Ladders** are stable at room temp. for 6 months



# 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](http://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?



IMPORTANT TIPS FOR Q5 HIGH-FIDELITY DNA POLYMERASE

### 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:

1. 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 T<sub>m</sub> values should be determined with NEB's T<sub>m</sub> Calculator (TmCalculator.neb.com)
- Primer pairs should have T<sub>m</sub> 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 T<sub>m</sub> values above 60°C
- To help eliminate primer degradation and subsequent non-specific product formation, use a hot-start enzyme (e.g., OneTaq Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase)

### Magnesium Concentration

- Optimal Mg<sup>2+</sup> concentration is usually 1.5–2.0 mM for most PCR polymerases
- Most PCR buffers provided by NEB already contain sufficient levels of Mg<sup>2+</sup> at 1X concentrations.
- NEB offers a variety of Mg-free reaction buffers to which supplemental Mg<sup>2+</sup> can be added for applications that require complete control over Mg<sup>2+</sup> concentration
- Further optimization of Mg<sup>2+</sup> 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 Mg<sup>2+</sup> in the reaction.
- Excess Mg<sup>2+</sup> may lead to spurious amplification; Insufficient Mg<sup>2+</sup> 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 Mg<sup>2+</sup> 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, OneTaq, 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., OneTaq 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., OneTaq 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 T<sub>m</sub> values should be determined using the NEB T<sub>m</sub> Calculator ([TmCalculator.neb.com](http://TmCalculator.neb.com))
- 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 T<sub>m</sub> 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 T<sub>m</sub> 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., OneTaq 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 T<sub>m</sub> of the primer pair
- Ideally, primer T<sub>m</sub> values should be less than the extension temperature. However, if T<sub>m</sub> 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

\*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.

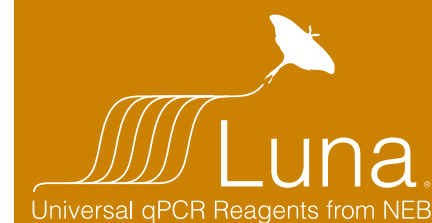


For more information on polymerase properties and usage, visit [www.neb.com](http://www.neb.com).

### qPCR & RT-qPCR GUIDELINES

Are you doing qPCR or RT-qPCR?

Visit [www.neb.com/qPCRguidelines](http://www.neb.com/qPCRguidelines) or [www.neb.com/RTqPCRguidelines](http://www.neb.com/RTqPCRguidelines) for optimization tips when using Luna qPCR & RT-qPCR products.





## PCR Polymerases

PRODUCT	NEB #	SIZE
Deep Vent DNA Polymerase	M0258S/L	200/1,000 units
Deep Vent (exo <sup>-</sup> ) 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>Taq</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 <i>Taq</i> Hot Start 2X Master Mix with GC Buffer	M0485S/L	100/500 reactions (50 µl reaction vol)
One <i>Taq</i> Hot Start Quick-Load 2X Master Mix with Standard Buffer	M0488S/L	100/500 reactions (50 µl reaction vol)
One <i>Taq</i> Hot Start Quick-Load 2X Master Mix with GC Buffer	M0489S/L	100/500 reactions (50 µl reaction vol)
One <i>Taq</i> 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)
<i>Taq</i> 2X Master Mix	M0270L	500 reactions (50 µl reaction vol)
<i>Taq</i> 5X Master Mix	M0285L	500 reactions (50 µl reaction vol)
<i>Taq</i> DNA Polymerase with Standard <i>Taq</i> Buffer	M0273S/L/X/E	400/2,000/4,000/20,000 units
<i>Taq</i> DNA Polymerase with Standard <i>Taq</i> (Mg-free) Buffer	M0320S/L	400/2,000 units
<i>Taq</i> DNA Polymerase with ThermoPol Buffer	M0267S/L/X/E	400/2,000/4,000/20,000 units
<i>Taq</i> PCR Kit	E5000S	200 reactions (50 µl reaction vol)
Vent DNA Polymerase	M0254S/L	200/1,000 units
Vent (exo <sup>-</sup> ) 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 [LUNAqPCR.com](http://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 $\mu$ mol of each
Deoxynucleotide Solution Mix	N0447S/L	8 $\mu$ mol of each/40 $\mu$ mol of each
dATP Solution	N0440S	25 $\mu$ mol
Acyclonucleotide Set	N0460S	0.5 $\mu$ mol of each
7-deaza-dGTP	N0445S/L	0.15 $\mu$ mol of each/0.3 $\mu$ mol of each
Ribonucleotide Solution Set	N0450S/L	10 $\mu$ mol of each/50 $\mu$ mol of each
Ribonucleotide Solution Mix	N0466S/L	8 $\mu$ mol of each/40 $\mu$ mol 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 $\mu$ g)	T1030S/L	50/250 preps
Exo-CIP™ Rapid PCR Cleanup Kit	E1050S/L	100/400 reactions



GERMANY & AUSTRIA  
New England Biolabs GmbH  
Brüningstr. 50, Geb B852  
65926 Frankfurt/Main, Germany  
Tel: +49/(0)69/305-23140  
Fax: +49/(0)69/305-23149

Free Call: 0800/246 5227 (Germany)  
Free Call: 00800/246 52277 (Austria)  
info.de@neb.com

[www.neb-online.de](http://www.neb-online.de)

#### HEADQUARTERS: USA

New England Biolabs, Inc.  
Telephone: (978) 927-5054

Toll Free (USA Orders): 1-800-632-5227  
Toll Free (USA Tech): 1-800-632-7799  
Fax: (978) 921-1350  
info@neb.com  
www.neb.com

[www.neb.com](http://www.neb.com)



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FRANCE  
New England Biolabs France SAS  
Genopole Campus 1, Bâtiment 6  
5 rue Henri Desbrères  
91030 Evry cedex, France  
Tel.: 0800 100 632 (Customer Service)

Tel.: 0800 100 633 (Technical Service)  
FAX.: 0800 100 610  
info.fr@neb.com

[www.neb-online.fr](http://www.neb-online.fr)

#### Your local NEB distributor:

BeNeLux:  
BIOKÉ  
info@bioke.com  
www.bioke.com

BULGARIA  
ELTA 90M Ltd.  
office@elta90.eu  
www.elta90.com

CROATIA:  
Diagnostica Skalpel d.o.o.  
skalpeli@skalpeli.hr  
www.skalpeli.hr

CZECH REPUBLIC:  
BioTech a.s.  
info@ibiotech.cz  
www.ibiotech.cz

DENMARK:  
BioNordika Denmark A/S  
info@bionordika.dk  
www.bionordika.dk

FINLAND:  
BioNordika Oy  
info@bionordika.fi  
www.bionordika.fi

GEORGIA  
Oxygen-Import, LLC  
info@oxygenimport.ge  
www.oxygenimport.ge

GREECE:  
BIOLINE SCIENTIFIC  
info@bioline.gr  
www.bioline.gr

HUNGARY:  
Kvalitex Kft.  
info@kvalitex.hu  
www.kvalitex.hu

ITALY:  
EUROCLONE S.P.A.  
info@euroclone.it  
www.euroclonegroup.it

ISRAEL:  
Ornat BioChemicals &  
Lab Equipment  
ornatbio@ornat.co.il  
www.ornat.co.il

KAZAKHSTAN  
INOS, LLC  
market@inos.kz  
inos.kz

LITHUANIA:  
Nanodiagnostika, Ltd.  
lina@nanodiagnostika.lt  
www.nanodiagnostika.lt

NORWAY:  
BioNordika AS  
info@bionordika.no  
www.bionordika.no

POLAND:  
Lab-JOT Ltd. Sp.z o.o. Sp.k.  
biuro@labjot.com  
www.labjot.com

PORTUGAL:  
Werfen  
dac-pt@werfen.com  
www.pt.werfen.com

ROMANIA:  
BioZyme  
contact@biozyme.ro  
www.biozyme.ro

SERBIA:  
Alfa Genetics d.o.o.  
office@alfagenetics.rs  
www.alfagenetics.rs

SPAIN:  
Werfen  
customerservice-es@werfen.com  
www.es.werfen.com

SLOVAK REPUBLIC  
BioTech s.r.o.  
info@ibiotech.sk  
www.ibiotech.sk

SLOVENIA:  
Mikro+Polo d.o.o.  
info@mikro-polo.si  
www.mikro-polo.si

SOUTH AFRICA:  
Inqaba Biotechnical Industries  
(Pty) Ltd  
info@inqababiotec.co.za  
www.inqababiotec.co.za

SWEDEN, ESTONIA, LATVIA,  
LITHUANIA:  
BIONORDIKA SWEDEN AB  
info@bionordika.se  
www.bionordika.se

SWITZERLAND:  
BioConcept Ltd.  
info@bioconcept.ch  
www.bioconcept.ch

TURKEY:  
EKA BIOLAB TEKNOLOJİ  
info@ekabiolab.com  
www.ekabiolab.com

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