Your commercial supply partner for innovation and productivity

OEM reagent guide for PCR, qPCR, and reverse transcription

ThermoFisher SCIENTIFIC

Contents

Innovation and quality to drive your ideas

We provide our partners with high-quality products and tailored support services from assay development strategy through manufacturing scale-up. Whether you are a large corporation or a start-up company, we offer you high-quality reagents with the security of stringent process control, allowing you to get breakthrough products to market faster and with less risk. The unique properties of our products help you succeed in the development and commercialization of nucleic acid–based tests with accuracy, reproducibility, and validity.

Leverage unique capabilities of our PCR reagents to drive your innovative solutions

- Custom functional testing tailored to meet your assay requirements
- High batch-to-batch consistency
- Sample availability for testing and validation
- Unparalleled bioinformatics capabilities, enabling 10M predesigned assays along with assay development capabilities

Integral quality systems are the cornerstone of our business

- ISO 9001– and ISO 13485–certified facilities
- Integral quality systems covering the supply chain, manufacturing, and quality control processes
- Raw material and vendor qualification to ensure consistent supply
- Rigorous documentation, complete traceability, and process control
- Adherence to comprehensive standard operating procedures (SOPs) to ensure consistency, compliance, and conformance of the products
- Openness to customer visits and audits

We provide long-term supply assurance

- Commercial supply and licensing agreements—securing long-term relationships
- Business continuity through contingency planning, risk management, and availability of multiple manufacturing sites
- Safe and efficient product delivery

We facilitate your product's path to market with tailor-made solutions to fit your applications and formats

- Comprehensive development and customization capabilities—custom product formulation, configuration, and quality control
- Supply of products at any scale and in any format—from bulk formats to finished goods
- Custom product labeling and packing capabilities with outer packaging—from a specific finish to the box design

We are your partner rather than your vendor

- Over 25 years in the OEM and commercial supply business
- Dedicated team of professionals to support your project
- Strict adherence to confidentiality obligations

Make us part of your team: contact our licensing and commercial supply specialist at **thermofisher.com/oem-partner**

Reverse transcription

Reverse transcriptases for RT-PCR and RT-qPCR applications

We offer a comprehensive portfolio of reverse transcriptases (RTs) from the wild type Moloney murine leukemia virus (M-MuLV) RT to the Invitrogen™ SuperScript™ line of RTs, with superior characteristics such as enhanced sensitivity and reduced reaction time (Table 1). Our proprietary technology of *in vitro* protein evolution has enabled the introduction of multiple favorable mutations into the traditional M-MuLV RT. This has dramatically improved the enzyme thermostability, resistance to inhibitors, and processivity. Our RT enzymes are also available in lyo-ready formulation (no glycerol; compatible with lyophilization), offering additional flexibility for RT-qPCR–based assay development.

Benefits

- Robust performance with challenging samples
- Enhanced thermostability and processivity
- High efficiency and sensitivity, even in the presence of inhibitors
- Minimal false-positive results with low residual host-cell DNA
- Lyophilization compatibility with lyo-ready formulation

Table 1. Reverse transcriptase selection chart.

Characteristic	SuperScript IV RT	SuperScript III RT	Maxima RT	RevertAid RT (M-MuLV)
Optimal reaction temperature	50˚C	50˚C	50˚C	42˚C
RNase H activity	No	No	Yes	Yes
RNase H minus version available	NA	NA	Maxima H Minus RT	RevertAid H Minus RT
Reaction time	10 min	50 min	30 min	60 min
Inhibitor resistance	++++	+	+++	+
Sensitivity	++++	+++	+++	++
Lyo-ready*	Yes	Yes	Yes	Yes

* Lyo-ready is a lyophilization-compatible enzyme composition without glycerol.
Note: "+" = poor; "++" = medium; "+++" = good; "++++" = recommended choice.

Featured reverse transcriptases

SuperScript IV Reverse Transcriptase

Invitrogen™ SuperScript™ IV RT is an M-MuLV RT mutant with superior robustness and reliability in cDNA synthesis (Figure 1). This RT enzyme exhibits strong inhibitor resistance, high processivity, thermostability, highly efficient full-length cDNA synthesis, and reduced RNase H activity.

SuperScript IV RT demonstrates its ability of reliable cDNA synthesis in the presence of common PCR reaction inhibitors, typical to situations where clinical or environmental samples of optimal quality are not available.

- Robust cDNA synthesis with a variety of gene targets, including degraded RNA and RNA samples of suboptimal purity
- High sensitivity (Figure 1) and linearity in a 10-minute reaction
- Linear dynamic range across a broad range of RNA input, crucial for detection of low-abundance samples

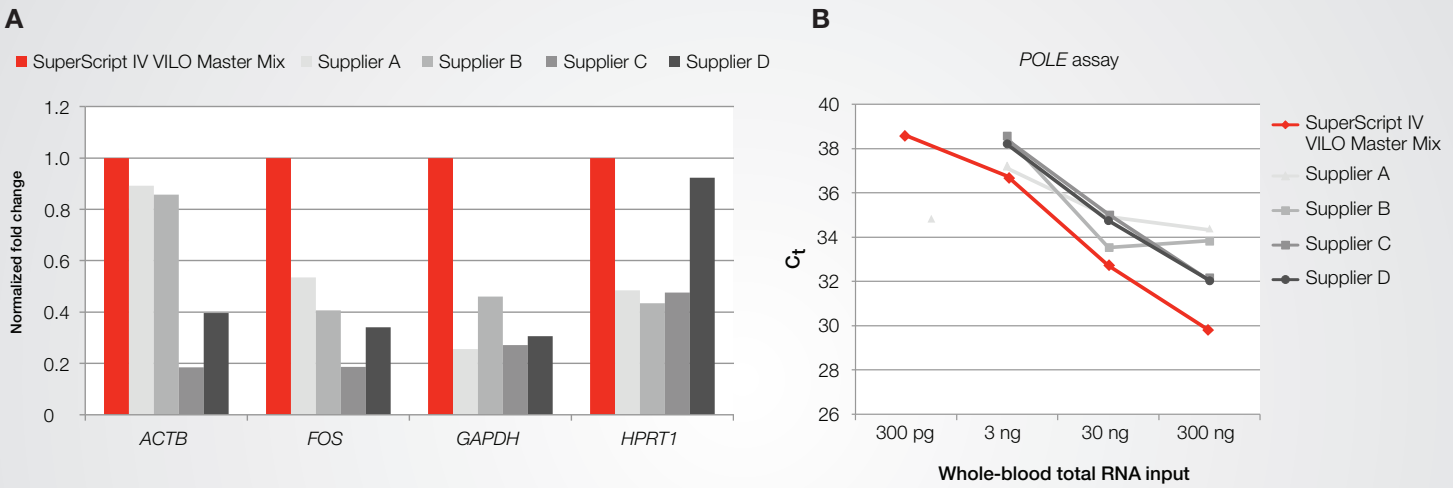


Figure 1. SuperScript IV RT provides higher sensitivity of target detection with whole-blood RNA. SuperScript IV and four other RTs (Suppliers A, B, C, and D) were used in RT-qPCR with 1 ng of partially degraded (RIN 4–6) blood RNA **(A)**, or with a dilution series of total whole-blood RNA **(B)**. Results in **(A)** are shown as normalized fold change relative to SuperScript IV RT, calculated as $2^{(C_t \text{ SuperScript IV RT} - C_t \text{ other product})}$.

SuperScript III Reverse Transcriptase

Widely used and with thousands of citations, Invitrogen™ SuperScript™ III RT offers higher cDNA yields and sensitivity than wild type M-MuLV RT enzymes.

- High thermostability for reduction of RNA secondary structures
- Reduced RNase H activity, delivering long RNA transcripts
- Robust enzyme with a half-life of 220 min at 50°C

Maxima Reverse Transcriptase

Thermo Scientific™ Maxima™ RT and Maxima™ H Minus RT are derived from *in vitro* evolution with unique attributes to maximize performance in cDNA synthesis.

- Reproducible cDNA synthesis and low variability levels across a wide range of starting RNA amounts
- Increased enzyme processivity for fast cDNA synthesis
- Tolerance to RT inhibitors

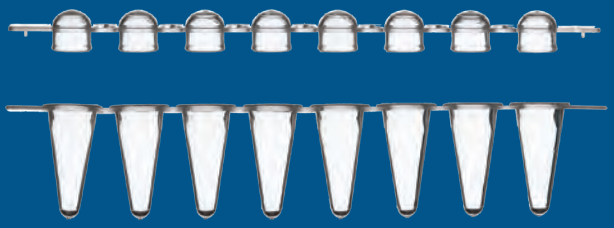
RevertAid Reverse Transcriptase

Thermo Scientific™ RevertAid™ RT is a wild type M-MuLV RT and offers routine performance of cDNA synthesis. Variants with and without RNase H activity are available.

Did you know?

Our plastic consumables are produced in injection-molding facilities that meet 10,000 or 100,000 (ISO 4 or ISO 5) clean room standards.

Learn more at **thermofisher.com/oemplastics**



Quality control

Quality testing is tailored to help ensure high performance of RTs in your RNA-based assays.

Parameter*	Method used
Unit concentration	One unit of the enzyme incorporates 1 nmol of dTMP into a polynucleotide fraction in 10 min at 37°C
Endo- and exodeoxyribonucleases	Incubation of radiolabeled, single- and double-stranded oligonucleotides with enzyme
Endodeoxyribonucleases (nicking activity)	Incubation of supercoiled plasmid DNA with enzyme
Ribonucleases	Incubation of radiolabeled RNA transcript with enzyme
Functional testing	Functionally tested in first-strand cDNA synthesis
Human gDNA*	Quantitative PCR test, which uses amplification of selected human gDNA fragments
E. coli gDNA*	Quantitative PCR test, which uses amplification of *E. coli* 23S rRNA gene fragment

* Scope of standard quality control program may vary for a specific enzyme.

DNA polymerases

Enhanced thermophilic DNA polymerases for qPCR and PCR applications

We are dedicated to providing PCR enzymes that meet the most demanding customer requirements. Our knowledge and expertise of enzymology and *in vitro* evolution allow us to develop enzymes with new or improved properties. Our customers can select enzymes from a spectrum of DNA polymerases based on a combination of hot-start technology, speed, level of resistance to PCR inhibitors, or lyophilization compatibility (Table 2). A high level of performance, lot-to-lot consistency, and extensive quality control testing enable the highest sensitivity, accuracy, and reproducibility in PCR and qPCR assays developed with our enzymes.

Benefits

- High sensitivity and specificity for detection of low-copy DNA targets
- Minimal activation time for faster sample to result
- Robust amplification of difficult-to-amplify targets, including those of suboptimal purity
- Lyophilization-compatible (lyo-ready) formulations available
- DNA-free DNA polymerase free from bacterial, animal, and human DNA contamination

Table 2. DNA polymerase selection chart.

Characteristics	Platinum *Taq* DNA Polymerase, inhibitor-resistant	Platinum *Taq* DNA Polymerase	AmpliTaq Gold DNA Polymerase	LibertyTaq DNA Polymerase	Wild type *Taq* DNA polymerase	Phire Hot Start II DNA Polymerase
Hot-start PCR	Antibody-based	Antibody-based	Chemically modified	Proprietary	No	Affibody-based
TaqMan probe–compatible	Yes	Yes	Yes	Yes	Yes	No
Reactivation time	2 min	2 min	10 min	0 min	0 min	0 min
Extension rate	15–30 sec/kb	30–60 sec/kb	30–60 sec/kb	30–60 sec/kb	30–60 sec/kb	10–15 sec/kb
Sensitivity	+++	+++	+++	+	+	+
Specificity	+++	+++	+++	+	+	++
Inhibitor resistance	+++	+	+	+	+	+++
Lyo-ready*	On request	Yes	On request	Yes	Yes	Yes
DNA-free**	On request	Yes	On request	On request	On request	On request

* Lyo-ready is a lyophilization-compatible enzyme composition without glycerol.

** DNA-free is enzyme manufactured using a single-use system to remove the risk of DNA contamination.

Note: "+" = poor; "++" = medium; "+++" = recommended choice.

Featured *Taq* DNA polymerases

Platinum *Taq* DNA Polymerase, inhibitor-resistant

Invitrogen™ Platinum™ *Taq* DNA Polymerase is derived from *in vitro* evolution, which allows for the development of unique characteristics, such as increased inhibitor resistance and enhanced speed. These attributes, combined with a robust hot-start feature, open new possibilities in DNA- and RNA-based assay design.

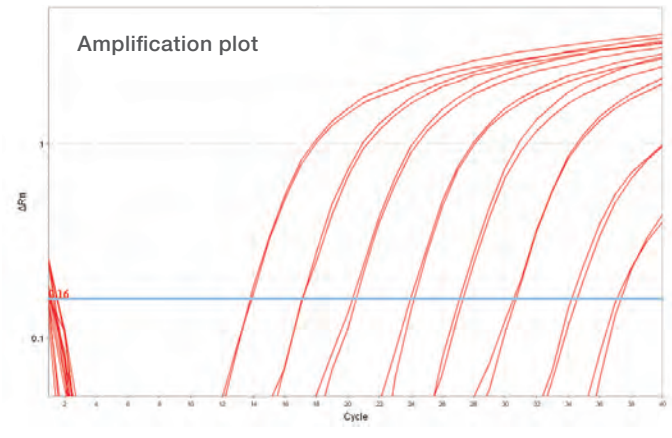
- Resistance to inhibitors present in challenging samples
- Shorter PCR cycling time for rapid amplification (Figure 2)
- Antibody-based hot-start technology delivers higher specificity, sensitivity, and yields (Figure 2)

Platinum *Taq* DNA Polymerase (also available in DNA-free format)

- Robust amplification of both low- and high-complexity DNA of different GC content
- 100x lower contaminating DNA than other "DNA-free" enzymes due to a single-use manufacturing system

LibertyTaq DNA Polymerase

- Innovative hot-start technology requires zero enzyme-activation time, with high specificity and sensitivity (Figure 3)
- Lyo-ready enzyme at a high concentration, without glycerol buffer



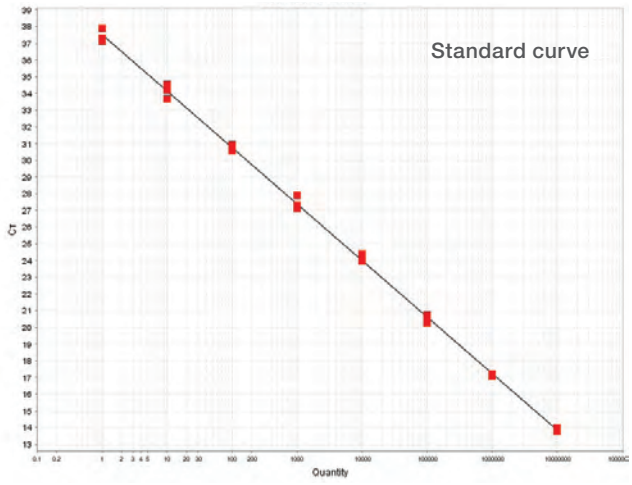


Figure 2. Fast and sensitive one-step RT-qPCR using Invitrogen™ Platinum™ *Taq* DNA Polymerase, inhibitor-resistant. Amplification of GAPDH RNA (from 1 x 10^7 to 1 copy) using Thermo Scientific™ Maxima™ Reverse Transcriptase (RT) and Platinum *Taq* DNA Polymerase, inhibitor-resistant in one-step RT-qPCR. GAPDH target was amplified using fast cycling conditions: 15 min at 50ºC (RT reaction); 3 min at 95ºC (RT inactivation); followed by 40 cycles of amplification (one cycle—15 sec at 60˚C).

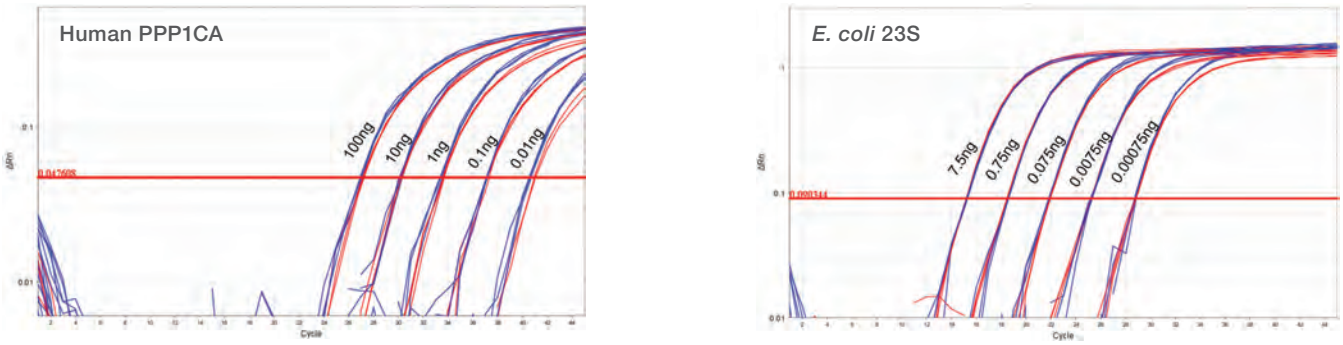
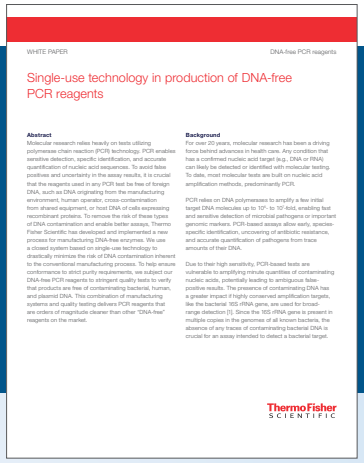


Figure 3. Sensitive, reproducible, and specific qPCR assays. Performance of lyo-ready Invitrogen™ LibertyTaq™ (blue curves) and Platinum *Taq* (red curves) DNA Polymerases were evaluated by qPCR using Applied Biosystems™ TaqMan® Assays for human PPP1CA and *E. coli* 23S ribosomal RNA (rRNA) genes and varying amounts of human or *E. coli* input genomic DNA (gDNA). Equally efficient and sensitive amplification was achieved with both DNA polymerases. No amplification was observed in no-template controls, confirming that formulations are free of contaminating human and *E. coli* DNA.

Did you know?

DNA contamination, which ranges from 10- to 1,000-genome equivalents of bacterial DNA/enzyme unit, is commonly found in commercially available enzymes. Check out the DNA-free PCR enzymes manufactured by a single-use system (SUS).

Download the white paper at **thermofisher.com/dna-free**


WHITE PAPER

DNA-free PCR reagents

Single-use technology in production of DNA-free PCR reagents

Abstract

Background

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Quality control

Quality testing tailored to ensure high performance of DNA polymerases in your DNA- and RNA-based assays.

Parameter*	Method used
Unit concentration	Incorporation of radiolabeled dNTP into polynucleotide fraction by enzyme during the selected time interval
Endo- and exodeoxyribonucleases	Incubation of radiolabeled, single- and double-stranded oligonucleotides with enzyme
Ribonucleases	Incubation of radiolabeled RNA transcript with enzyme
Residual activity assay	Extension of labeled, double-stranded oligonucleotides by enzyme without heat reactivation
Functional testing	Quantitative PCR containing 10-fold dilutions over five orders of magnitude of human and *E. coli* gDNA
Human gDNA	Quantitative PCR test, which uses amplification of selected human gDNA fragments
E. coli gDNA	Quantitative PCR test, which uses amplification of an *E. coli* 23S rRNA gene fragment

* Scope of standard quality control program varies for different enzymes. Quality control methods for Applied Biosystems™ AmpliTaq Gold™ DNA Polymerase differ from the ones described above.

Proofreading enzymes

The gold-standard for high-fidelity applications

Invitrogen™ and Thermo Scientific™ High-Fidelity DNA Polymerases are designed to amplify DNA fragments with exceptional robustness and fidelity, and to generate PCR products with high accuracy and speed even for the most difficult templates. Choose from a collection of our high-fidelity enzymes, their formats, buffers, and dNTP solutions, depending on the sophistication of your DNA-based assay and your needs for flexibility (Table 3).

Benefits

- Highest fidelity on the market (>100x *Taq* polymerase)
- Robust amplification of versatile targets
- Exceptional tolerance to PCR inhibitors
- Shorter cycling times for faster sample to result
- High yields without optimization
- Minimized nonspecific amplification
- Available as a stand-alone enzyme or in a master mix format

Table 3. High-fidelity DNA polymerase selection chart.

Characteristics	Platinum SuperFi DNA Polymerase	Phusion Hot Start II High-Fidelity DNA Polymerase	Phusion U Hot Start DNA Polymerase	Phusion High-Fidelity DNA Polymerase
Fidelity vs. *Taq* polymerase	>100x	52x	25x	52x
Hot-start PCR	Antibody-based	Affibody-based	Affibody-based	No
Target length	≤20 kb	≤20 kb	≤20 kb	≤20 kb
Extension rate	15–30 sec/kb	15–30 sec/kb	15–30 sec/kb	15–30 sec/kb
TaqMan probe–compatible	No	No	No	No
Inhibitor resistance	++++	+++	++	++
dUTP tolerance	No	No	Yes	No
Multiplexing	Yes	Yes	Yes	No
Lyo-ready*	On request	Yes	On request	On request

* Lyo-ready is a lyophilization-compatible enzyme composition with low glycerol (<1%).
Note: "++" = medium; "+++" = recommended choice; "++++" = outstanding.

Featured high-fidelity DNA polymerases

Platinum SuperFi DNA Polymerase

Invitrogen™ Platinum™ SuperFi™ DNA Polymerase is designed for success in PCR, combining the highest fidelity with trusted Platinum™ hot-start technology. Featuring >100x *Taq* fidelity, Platinum SuperFi DNA Polymerase is ideally suited for applications benefiting from supreme sequence accuracy.

- Exceptionally high fidelity
- High specificity and increased yields with Platinum hot-start technology
- Robust amplification of a wide range of targets with resistance to common PCR inhibitors
- Convenient workflow with room temperature reaction setup

The Platinum SuperFi DNA Polymerase provides the highest level of confidence for preserving DNA sequence accuracy with its extremely low error rate (>100x higher fidelity than *Taq* polymerase, as shown in Figure 4).

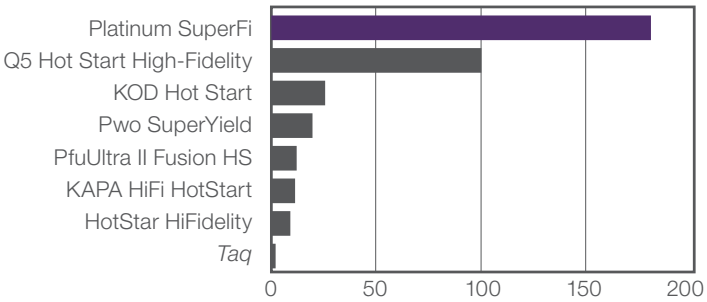


Figure 4. Relative fidelity values of different DNA polymerases. The fidelity of DNA polymerases was measured by next-generation sequencing. The background level of experimental errors was estimated from PCR-free library sequencing data. The polymerase fidelities were normalized to *Taq* polymerase. It is difficult to determine the fidelity values that are greater than 100x *Taq* polymerase in a statistically significant manner, because the extremely low error rates are at the background level.

Resistance to inhibitors

Platinum SuperFi DNA Polymerase is engineered with a DNA-binding domain exhibiting high processivity and increased resistance to common PCR inhibitors, such as heparin, xylan, and humic acid (Figure 5).

Figure 5. Resistance to inhibitors. Amplification of a 2 kb human gDNA fragment using Platinum SuperFi DNA Polymerase or high-fidelity DNA polymerases from other suppliers (A–D) in reaction mixtures containing 1 = no inhibitor, 2 = heparin (0.15 µg/µL), 3 = xylan (0.5 µg/µL), or 4 = humic acid (0.5 ng/µL).

Since the introduction of Thermo Scientific™ Phusion™ High-Fidelity DNA Polymerase in 2003, a new standard of performance was established for high-fidelity PCR. During the last decade, our high-fidelity DNA polymerases have been proven significantly useful in demanding PCR applications, including the creation of the first functional synthetic genome.

Using a unique fusion technology, with a DNA-binding domain fused to a *Pyrococcus*-like proofreading polymerase, Phusion DNA polymerases generate PCR products with very high accuracy and speed. In addition, Phusion DNA polymerases are tolerant of various inhibitors—allowing robust amplification of PCR products with minimal optimization.

Phusion Hot Start II High-Fidelity DNA Polymerase

With a combination of hot-start and high-fidelity PCR, Thermo Scientific™ Phusion™ Hot Start II High-Fidelity DNA Polymerase is an ideal choice, allowing high specificity and improved robustness (Figure 6).

- High fidelity (52x higher fidelity than *Taq* polymerase, 6x more accurate than *Pfu* polymerase)
- Increased processivity allows for shorter reaction times
- Exceptional product yields with minimal enzyme amounts
- Enhanced specificity with unique affibody ligand–based hot-start technology with no time required for reactivation

Figure 6. Direct PCR amplification of a blood sample using Phusion Hot Start II High-Fidelity DNA Polymerase. Phusion Hot Start II High-Fidelity DNA Polymerase, as part of the Thermo Scientific™ Phusion™ Blood Direct PCR Kit, was compared to other kits designed for performing PCR directly from blood. A 588 bp genomic DNA fragment was amplified in the presence of increasing blood concentrations in the reaction mixture. PCR was performed according to the suppliers' instructions. Total protocol times are indicated at the bottom. Positive- and negative-control reactions are denoted by "+" and "–", respectively.

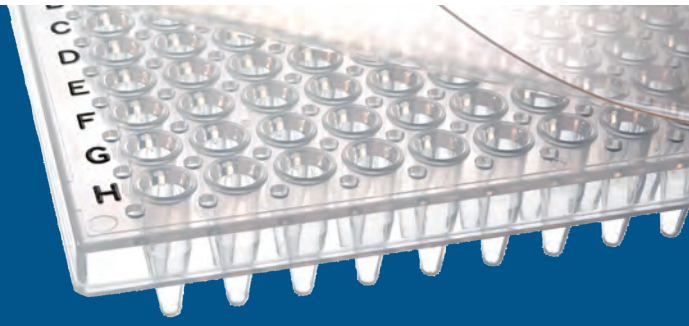
Phusion U Hot Start DNA Polymerase
The Thermo Scientific™ Phusion™ U Hot Start DNA Polymerase is a high-fidelity DNA polymerase engineered for uracil-tolerant PCR. Phusion U Hot Start DNA Polymerase carries a mutation in the dUTP-binding pocket of the Phusion enzyme and is tolerant to dUTP present in DNA templates and able to incorporate dUTP.

- Engineered high-fidelity polymerase for uracil-tolerant PCR
- High accuracy (25x higher fidelity than *Taq* polymerase)
- High processivity allows for shorter reaction times
- Ideal for PCR-based assays with the need for carryover contamination control

Did you know?

Off-the-shelf plastics options are available for many instruments, including Applied Biosystems™ thermal cyclers and real-time PCR instruments, PCR tubes, strip tubes, 24- to 384-well PCR plates, and a wide range of plate seals.

Learn more at **thermofisher.com/oemplastics**



Quality control

Quality of high-fidelity DNA polymerases is ensured by extensive quality testing.

Parameter*	Method used
Unit concentration	One unit of enzyme incorporates 10 nmol of dNTPs into a polynucleotide fraction at 74ºC in 30 min
Endodeoxyribonucleases (nicking activities)	Incubation of supercoiled plasmid DNA with 10 U of enzyme at 37ºC for 4 hr and analysis on agarose gel
Residual activity assay	Extension of labeled, double-stranded oligonucleotide with 5′-overhangs after incubation for 4 hr at 37°C in presence of dNTPs
Functional testing in PCR	PCR amplification of 7.5 kb fragments from human gDNA and 20 kb fragments from lambda DNA, and analysis on agarose gel
Human gDNA	Quantitative PCR test, which uses amplification of Alu repeats in human gDNA, performed on DNA purified from enzyme

* Scope of standard quality control program varies for different enzymes.

qPCR-grade dNTPs

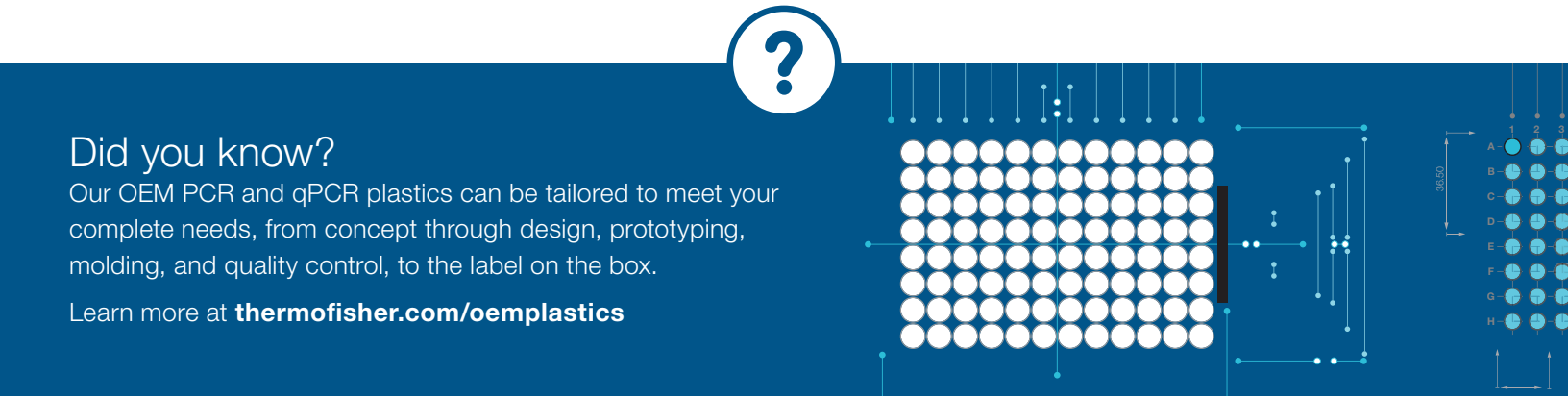
Outstanding performance and reliability for the most demanding applications

Our dNTPs have been extensively tested and verified for use in a wide variety of molecular biology applications, including highly sensitive techniques such as RT-qPCR (Figure 7) and next-generation sequencing.

All dNTP formulations are designed for convenience and flexibility. Standard nucleotides (dATP, dCTP, dGTP, dTTP, and dUTP) are supplied as 100 mM solutions, and nucleotide mixes can be formulated up to a 100 mM concentration for each nucleotide in the mix.

Benefits

- Manufactured using dedicated equipment for each dNTP
- Tested to be free of contaminating RNase or DNase activities and inhibitors of qPCR, PCR, and reverse trancription
- High stability—36-month shelf life; remains stable after >100 freeze-thaw cycles
- Large-scale manufacturing (>1,000 L)
- Available as individual dNTP solutions and mixes
- High purity (≥99%) according to HPLC (Figure 8)



Did you know?

Our OEM PCR and qPCR plastics can be tailored to meet your complete needs, from concept through design, prototyping, molding, and quality control, to the label on the box.

Learn more at **thermofisher.com/oemplastics**

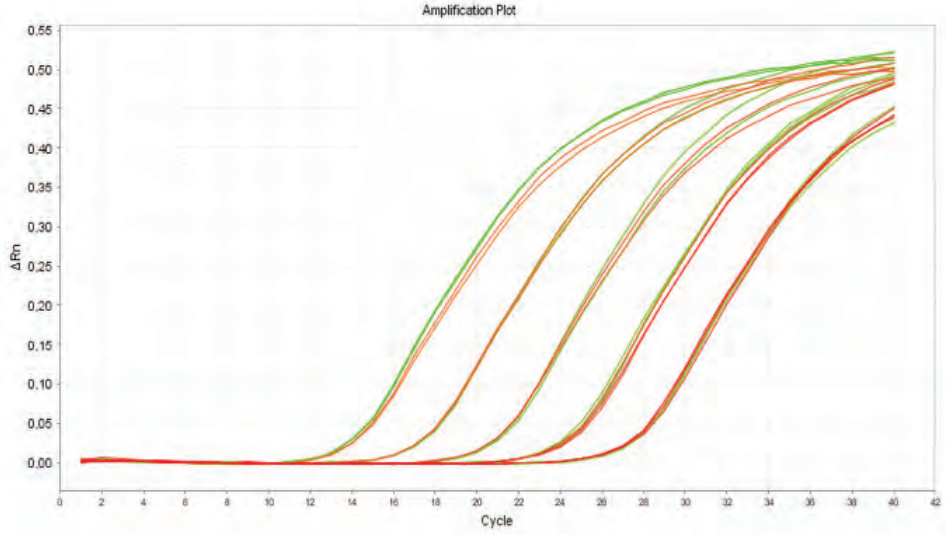


Figure 7. High quality of dNTPs ensures consistent C_t values in qPCR, especially at low-input template concentrations. dNTPs are tested for detection of the *GAPDH* gene in two-step RT-qPCR using different amounts of RNA transcript (0.05 µg/µL RNA solution diluted from 1 x 10^7 to 1 x 10^3 copies) in reverse transcription reactions followed by amplification with hot-start *Taq* DNA polymerase.

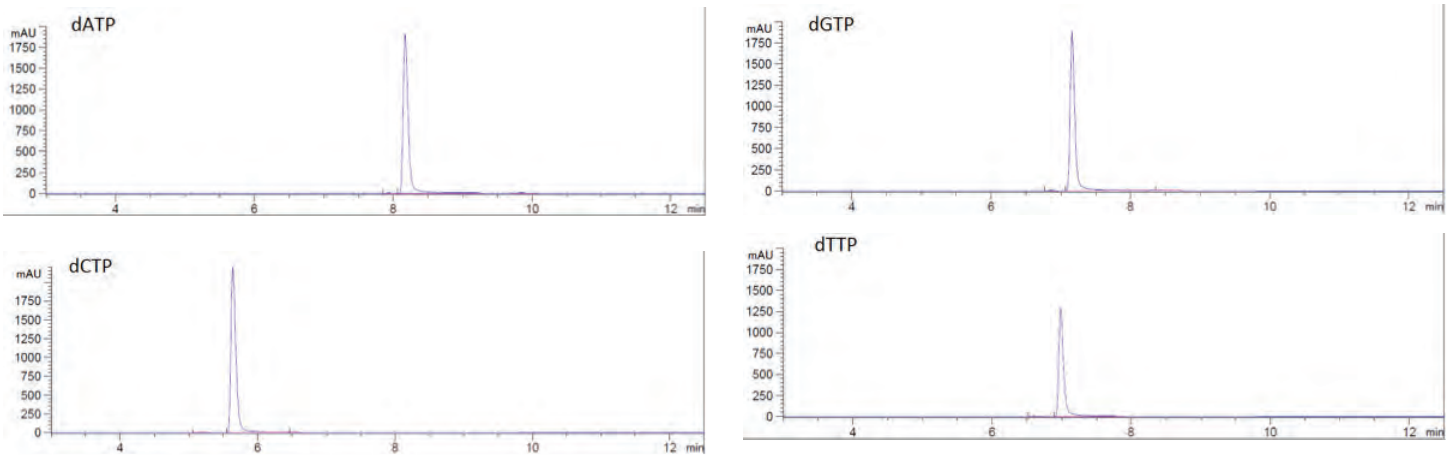


Figure 8. Relative HPLC profiles of ≥99% pure dNTPs. HPLC analysis shows greater than 99% triphosphate purity with undetectable mono-, di-, and tetraphosphate forms.

Quality control

Quality testing is tailored to help ensure high performance of dNTPs in your DNA- and RNA-based assays.

Parameter*	Method used
Appearance	Clear, colorless solution
pH value	7.3–7.5
Concentration	100 ±3 mM
Base purity (HPLC)	>99.5% deoxynucleoside
Purity (HPLC)	≥99% triphosphate
Pyrophosphate	<0.003 pmol PPi/pmol dNTP
Endodeoxyribonuclease and nicking activity	Undetectable after incubation of supercoiled plasmid DNA with dNTP
Endo- and exodeoxyribonucleases	Undetectable after incubation of radiolabeled, single- and double-stranded oligonucleotides with dNTP
Ribonucleases	Undetectable after incubation of RNA transcript with dNTP
Human DNA	Undetectable in quantitative PCR test, which uses amplification of Alu repeats in human gDNA
E. coli DNA	Undetectable in quantitative PCR test, which uses amplification of an *E. coli* 23S rRNA gene fragment
Functional test	Functionally tested in two-step RT-qPCR using different starting amounts of RNA transcript in reverse transcription reactions followed by amplification with hot-start *Taq* DNA polymerase

* Scope of standard quality control program may vary for custom products.

Real-time PCR master mixes

Proven performance in qPCR

Applied Biosystems™ TaqMan® and TaqPath™ master mixes provide turnkey solutions for real-time PCR. They contain buffer, dNTPs, passive reference dye, thermostable hot-start DNA polymerase, and other components formulated for reliable 5′ nuclease probe-based real-time PCR. Just add your sample and TaqMan assay components, and start your reactions (Table 4).

We offer a range of real-time PCR master mixes optimized for specific applications, and provide reagents for Research Use Only (RUO) and general purpose reagents (GPRs) for laboratory use.

TaqPath qPCR master mixes have been validated to deliver lot-to-lot reproducibility for absolute consistency in C_t values and dynamic range, required by molecular diagnostics developers. These GPRs are manufactured in an ISO 13485 facility and include control measures, change control, document control, and purchasing controls. In addition, each batch is quality control–tested prior to release. TaqPath qPCR master mixes enable superior performance, improved reproducibility, and traceability.

Table 4. Features of TaqPath master mixes.

Application	Name	GPR*	Passive reference	Features/advantages
Genotyping and copy number variation (CNV)	TaqPath ProAmp Master Mix and ProAmp Multiplex Master Mixes	Yes	ROX, Mustang Purple	Accurate genotyping calls in the presence of inhibitors
DNA detection and 2-step gene expression	TaqPath qPCR Master Mix	Yes	ROX	Linear, reproducible results over a wide dynamic range
RNA virus detection and 1-step gene expression	TaqPath 1-Step RT-qPCR Master Mix and Multiplex Master Mix	Yes	ROX, Mustang Purple, or None	Sensitive, reproducible detection, even in the presence of inhibitors
2-step gene expression and DNA detection	TaqMan Fast Advanced Master Mix and TaqMan Multiplex Master Mix	No	ROX	Wide linear dynamic range with reduced runtimes
RNA virus detection and 1-step gene expression	TaqMan Fast Virus 1-Step Master MIx	No	ROX	Sensitive and linear results, even in the presence of inhibitors

* For laboratory use. General purpose reagent.

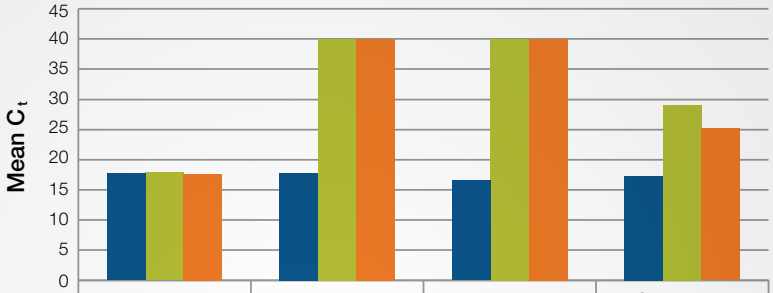
Featured master mix

TaqPath 1-Step Multiplex Master Mix

Developed for RNA virus detection and high-throughput gene expression analysis protocols, Applied Biosystems™ TaqPath™ 1-Step Multiplex Master Mix delivers sensitive and reproducible detection of up to four RNA (or DNA) targets in a single multiplex RT-qPCR reaction. While the single-tube, 4X format facilitates easy reaction setup, the reproducible performance, even in the presence of inhibitors, provides confidence in results.

Features of TaqPath 1-Step Multiplex Master Mix:

- Tolerance of inhibitors commonly found in clinical samples (Figure 9)
- Ability to detect up to four targets in one reaction
- High sensitivity to detect low-copy targets with reproducible C_t values
- Wide dynamic range compatible with multiplexing applications
- Manufactured with stringent process controls to help ensure lot-to-lot consistency



	TaqPath 1-Step Multiplex Master Mix	iTaq Universal Probes One-Step Kit	QuantiTect Virus Kit	qScript XLT One-Step ToughMix master mix
No inhibitor	17.6	17.6	16.6	17.3
10 µM hematin	17.9	40.0	40.0	29.0
0.0625 U/µL heparin	17.6	40.0	40.0	25.2

Figure 9. Inhibitor tolerance of TaqPath 1-Step Multiplex Master Mix and kits from other suppliers. Two inhibitors (hematin and heparin) were added to RT-qPCR reactions run on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System to assess the magnitude of C_t shift caused by these inhibitors. C_t values for reactions without and with inhibitors are shown.

Real-time PCR assays

Proven performance in qPCR

TaqMan MGB probes

Applied Biosystems™ TaqMan® probes include an minor groove binding (MGB) moiety at the 3′ end that increases the T_m of the probe and stabilizes probe-target hybrids. This means that MGB probes can be significantly shorter than the traditional probes, which can provide flexibility in designing probes for molecular test development.

TaqMan MGB probes incorporate a nonfluorescent quencher (NFQ) to absorb (quench) signal from the fluorescent dye labeled at the other end of the probe. The properties of the NFQ combined with the short length of the MGB probe result in lower background signal than with non-MGB NFQ probes. Lower background signal means increased sensitivity and precision in your data (Figure 10).

MGB probes and unlabeled primers are also available as analyte specific reagents (ASRs) to help meet requirements for test development. Applied Biosystems™ Probe and Primer ASRs can be used across a wide variety of applications, including gene expression, genotyping, and copy number analysis.

The Probe and Primer ASRs are manufactured in a registered facility in accordance with current good manufacturing practices (cGMPs) with a quality management system certified to ISO 13485:2003.

TaqMan probe outperforms non-MGB probe in real-time PCR—in dynamic range, sensitivity, and reproducibility

	C_t values		Standard deviation	
Input	TaqMan assay	Non-MGB assay	TaqMan assay	Non-MGB assay
10 ng	9.72	13.35	0.02	0.15
1 ng	13.36	16.82	0.04	0.18
0.1 ng	16.76	20.23	0.07	0.13
10^{-2} ng	20.19	23.72	0.04	0.13
10^{-3} ng	23.64	27.31	0.03	0.10
10^{-4} ng	27.01	30.66	0.04	0.12
10^{-5} ng	30.24	32.82	0.13	0.19

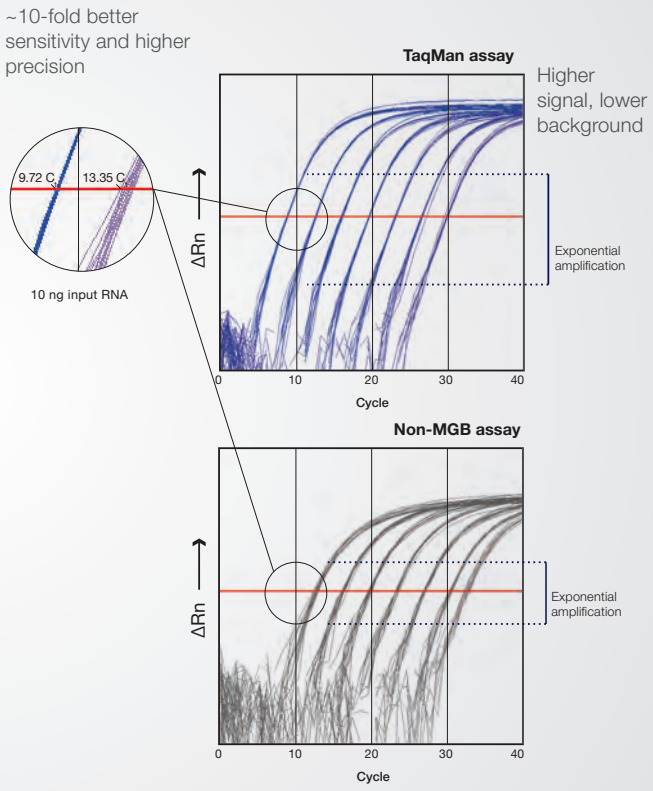


Figure 10. TaqMan probes provide better sensitivity and precision. Comparison of two 5′ nuclease PCR assays for 18S rRNA. Ten-fold dilutions of Universal Human Reference RNA (10–10^{-5} ng) were prepared and analyzed in 11 replicate real-time PCR reactions using either the Applied Biosystems™ TaqMan® Gene Expression Assay (FAM dye–labeled, with NFQ) or the non-MGB assay (FAM dye–labeled, with BHQ). Real-time PCR was run according to the respective manufacturers' recommended conditions. Across a 6-log range of input template, the TaqMan assay displayed lower C_t values and better reproducibility across all data points. In addition, the TaqMan assay had higher signal and lower background, resulting in better sensitivity and higher precision.

TaqMan QSY probes for multiplexing

Applied Biosystems™ TaqMan® QSY™ probes incorporate a proprietary 3′ QSY quencher to provide maximal PCR performance in a multiplex format. TaqMan QSY probes are available with FAM™, VIC™, and our proprietary ABY™ and JUN™ dyes, allowing amplification of up to four targets in a single reaction. All four dyes are optimized for the filter sets on Applied Biosystems™ real-time PCR instruments and work together with minimal spectral overlap for optimal performance (Figure 11).

Multiplexing with TaqMan QSY probes enables cost savings and preservation of limited samples, and also yields comparable results between reactions performed in individual tubes or in 4-plex reactions for a gene quantification experiment (Figure 12).

TaqMan real-time PCR assays

Applied Biosystems™ TaqMan® assays are optimized primer and probe sets in one convenient tube. We provide a complete range of custom-synthesized oligonucleotide primers and probes, all built to your specifications. Simply identify your target of interest or exact sequence, and our team can design your assay. In addition to custom oligos, we also offer predesigned primer and probe sets for our TaqMan assays, developed by our in-house bioinformatics team.

Choose from over 10 million predesigned assays or design your custom assays across multiple applications:

- Gene expression
- SNP genotyping
- Copy number
- MicroRNA
- Mutation detection

Benefits

- Advanced primer and probe sequence selection criteria generate highly specific amplification and detection of your target
- The NFQ on TaqMan® probes minimizes background—reliably detects targets present at 10 or fewer copies
- Reproducible results from well to well, day to day, and lab to lab—even across manufacturing lots
- Wide dynamic range from a handful to millions of target molecules with the same reaction setup
- All assays use a single, universal thermal cycling profile

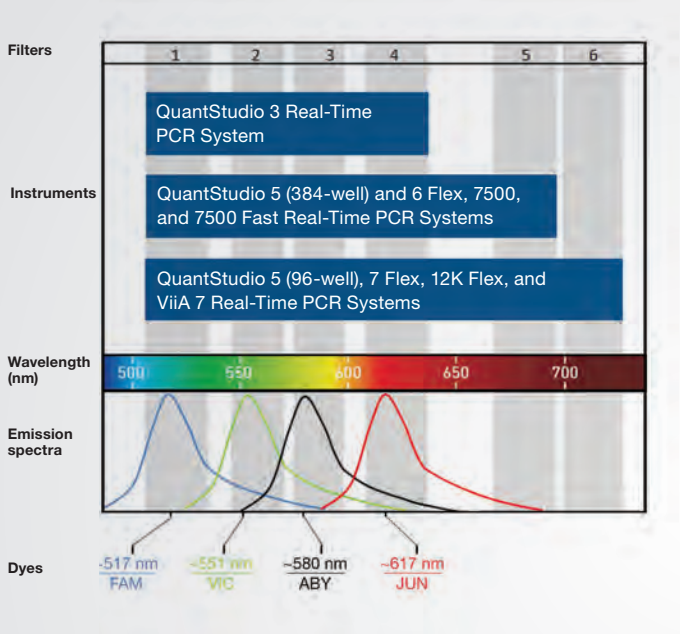


Figure 11. Fluorescence emission wavelengths used for multiplex real-time PCR. Emission spectra for FAM, VIC, ABY, and JUN dyes are shown in relation to regions of the spectrum detected by six filters available on Applied Biosystems real-time PCR instruments.

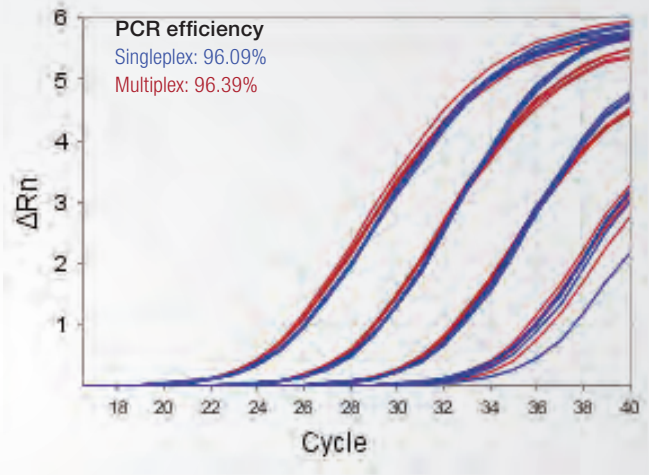


Figure 12. Comparable results for singleplex and multiplex assays. The amplification plot shows linear portions of the curves for 4 EGFR assays amplified in singleplex (blue) and 4-plex reactions (red) in a dilution series from 20,000 pg to 2 pg of reference colon cDNA per 10 µL reaction. PCR efficiencies are 96.09% for EGFR singleplex and 96.39% for EGFR 4-plex reactions.

Customer service

Maintaining long-term relationships with partners is our goal; understanding your needs, challenges, and requirements is the foundation of our business. We communicate openly and deliver on our promises.

- Your business will be assigned to a dedicated team of experts that specialize in OEM
- We provide prompt and accurate responses to questions, documentation requests, and technical support
- We take your confidentiality very seriously

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