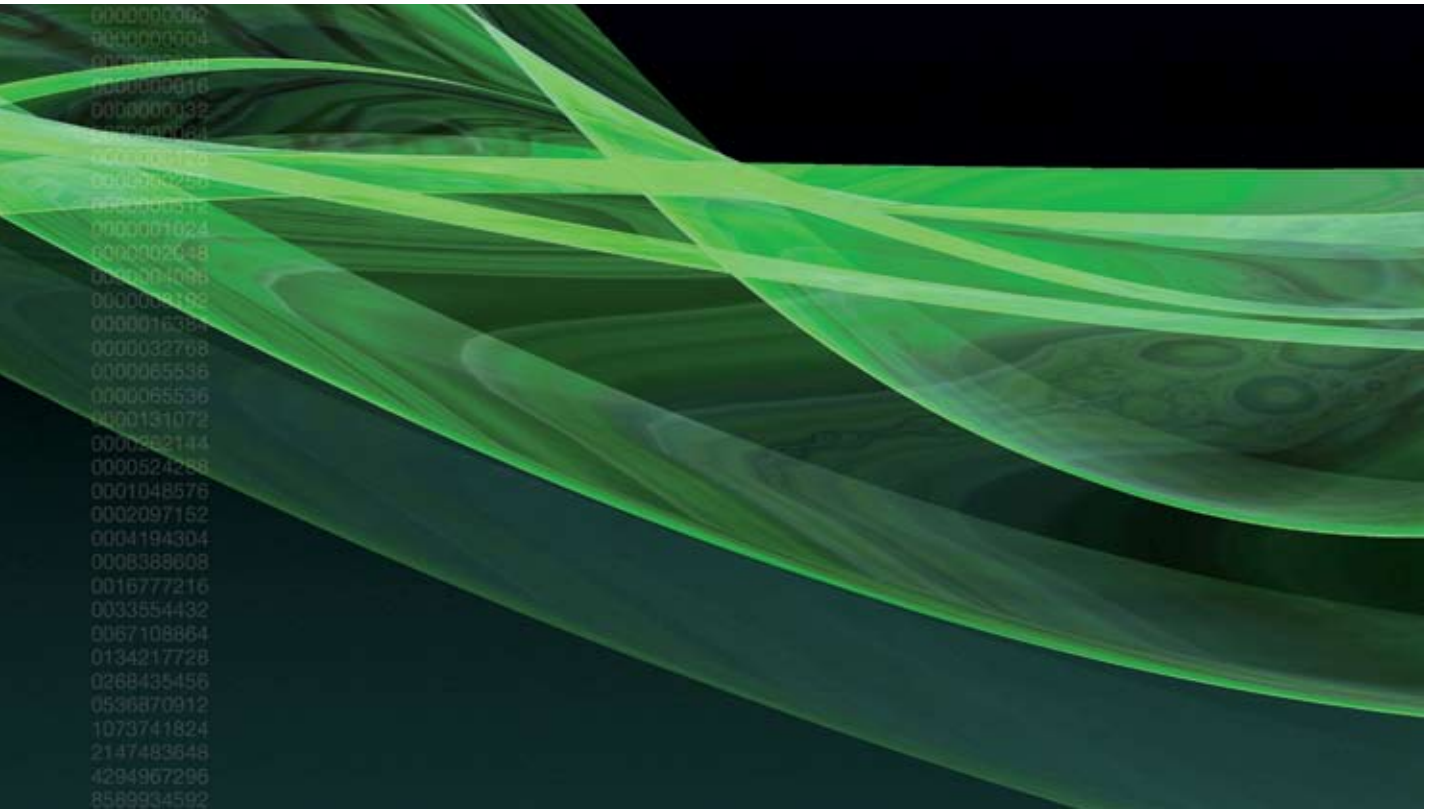


Amplification: PCR Reagents



Reagents

For Reverse Transcription, PCR, and Real-Time PCR

BIO-RAD

iScript cDNA Synthesis Kit

Preblended Primers for Optimal Performance

With only three tubes, the iScript cDNA synthesis kit is the easiest to use and most sensitive system available for first-strand cDNA synthesis. This rigorously optimized kit features a 5x iScript reaction mix containing a blend of oligo(dT) and random primers for unbiased representation over a broad range of input RNA amounts. With the iScript cDNA synthesis kit, achieving great results has never been easier.

- MMLV RNase H⁺ reverse transcriptase delivers the highest sensitivity for real-time RT-PCR
- Optimized blend of oligo(dT) and random primers enables complete and unbiased RNA sequence representation
- Easy reaction assembly and streamlined protocol greatly simplify reverse transcription reactions

iScript Select DNA Synthesis Kit

Superior Performance Using a Flexible Selection of Primers

Appropriate for a variety of applications, including real-time RT-PCR and creation of cDNA fragments >6 kb, this sensitive cDNA synthesis kit offers simplified handling and superior performance. With a flexible format designed to accommodate a variety of priming strategies, this kit provides optimized reagents and protocols for oligo(dT), random, or custom-designed gene-specific primers.

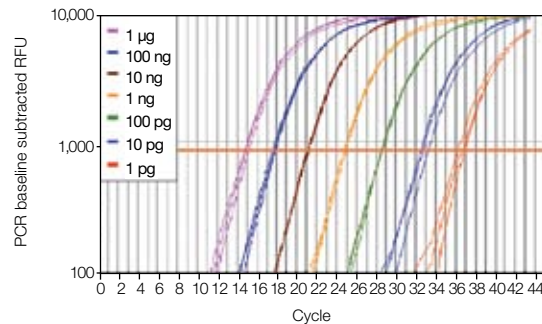
- MMLV RNase H⁺ reverse transcriptase for sensitive detection using 1 µg to 1 pg of input total RNA
- Sensitive and consistent performance using your choice of oligo(dT), random, or gene-specific primers
- Quality controlled for reliable synthesis of cDNA >6 kb

iScript One-Step Quantitative RT-PCR Kits

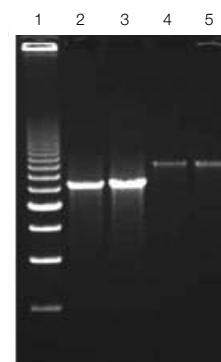
Convenient One-Step qRT-PCR for Any Detection Chemistry

The iScript one-step RT-PCR kits are optimized to deliver maximum RT-PCR efficiency, sensitivity, and specificity. Both kits contain a proprietary reaction buffer that has been specifically formulated to optimize activity of both iScript reverse transcriptase and iTaq DNA polymerase, while minimizing the potential for primer-dimer formation and other nonspecific PCR artifacts. Thus, with these kits, clean detection of low-copy targets is easy to achieve.

- Highly specific amplification over a broad dynamic range, using either SYBR Green or probe-based detection chemistries
- Extremely sensitive detection, down to 100 fg of input RNA
- Convenient one reaction setup that minimizes handling and contamination risk

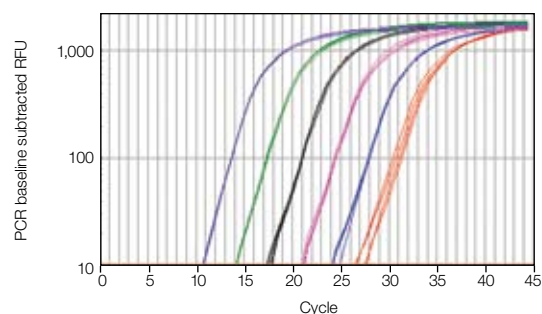


The iScript cDNA synthesis kit performs across a broad range of concentrations. Input RNA was reverse transcribed, amplified using iQ SYBR Green supermix, and detected on the iCycler iQ[®] real-time PCR detection system. Bio-Rad's iScript cDNA synthesis kit delivered a dynamic range of 6 orders of magnitude while maintaining optimum sensitivity with limited amounts of input RNA. Standard curve had $r = 0.998$ and efficiency = 96.5%.



The iScript Select cDNA synthesis kit facilitates synthesis of cDNA longer than 6 kb.

First-strand cDNA was produced from 1 µg total RNA using the iScript Select cDNA synthesis kit and the provided oligo(dT) primer mix. A 2 µl aliquot of the 20 µl cDNA synthesis reaction was subjected to 35 PCR cycles using a proofreading high-fidelity polymerase and human adenomatous polyposis coli (APC) primer sets. From a 50 µl PCR reaction, 10 µl was analyzed on a 1% agarose gel. Lane 1, 1 kb ladder; lanes 2 and 3, 6.4 kb APC PCR product; lanes 4 and 5, 8.2 kb APC PCR product.



The iScript one-step RT-PCR kit with SYBR Green provides high sensitivity across a broad range of concentrations. One-step RT-PCR reactions were performed in triplicate, along with no-template controls, using GAPDH primers and 100 ng to 100 fg of total HeLa RNA. Reactions were carried out on the iCycler iQ real-time detection system. Standard curve had $r = 1.000$ and efficiency = 95%.

iQ Supermixes

Sensitive Supermixes for Real-Time PCR

Our versatile iQ supermixes provide the ultimate convenience in preblended solutions for a wide array of real-time PCR applications. The iQ SYBR Green supermix also contains fluorescein for the collection of well factors on most Bio-Rad real-time PCR detection systems.

- iTaq hot-start DNA polymerase allows sensitive and accurate detection of as few as 10 copies of template
- 2 unique formulas afford compatibility with any fluorescent detection chemistry, including both sequence-specific probes and SYBR Green I
- Robust supermixes provide precise linear detection over 6 orders of magnitude

iTaq Supermixes With ROX

Supermixes for All ROX-Dependent Real-Time Thermal Cyclers

The iTaq supermixes with ROX are formulated to easily achieve optimal results in real-time quantitative PCR assays. These mixes yield high performance over a broad dynamic range, achieving sensitive and specific amplification over at least 6 orders of magnitude.

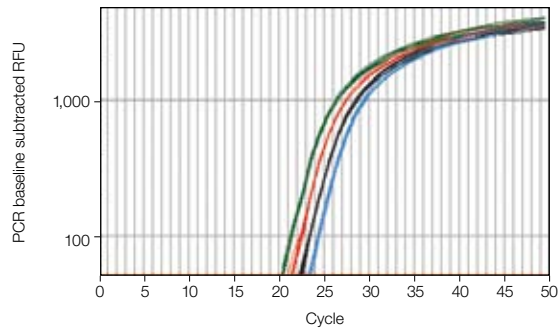
- Sensitive and accurate detection of low-abundance targets
- Convenient 1-tube formulation, preblended with ROX to correct for interwell signal variation
- Unique formulas afford compatibility with any fluorescent detection chemistry on ROX-dependent platforms, including fast-cycling instruments

iQ Multiplex Powermix

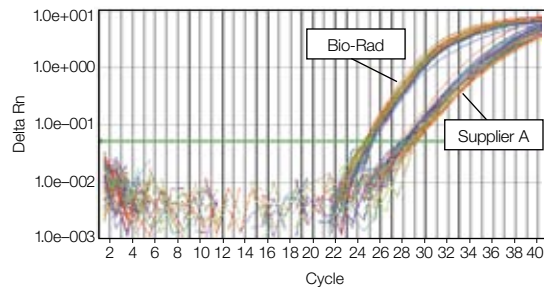
Detect Multiple Targets Without Optimization

Bio-Rad has applied its expertise in multiplex real-time PCR to create a robust mix that greatly simplifies real-time detection of multiple targets in a single tube. Finding a set of reaction conditions that amplifies all targets with equal efficiency in both singleplex and multiplex reactions can be a challenge. To help simplify multiplex real-time PCR, we have developed the iQ multiplex powermix. This mix makes multiplex real-time PCR easier by removing the need to optimize buffer, enzyme, or primer concentrations.

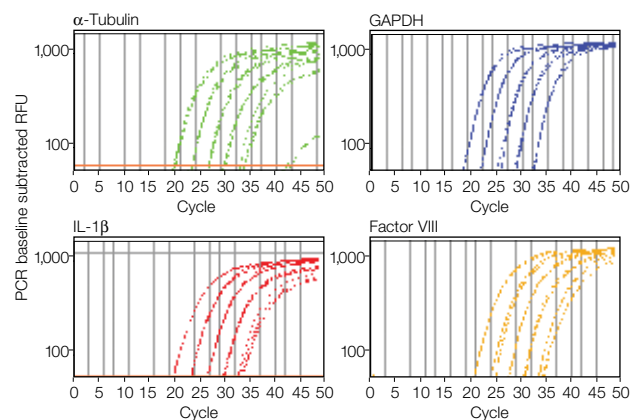
- Reliable real-time multiplex detection of up to 5 targets
- Detection of up to 4 targets, when one differs in expression up to 10^6 -fold relative to the others
- Linearity over 6 orders of magnitude of input cDNA and 4 orders of magnitude of input genomic DNA



An accurate one-cycle spacing in C_t is precisely maintained in a series of 2-fold dilutions. Human genomic DNA was amplified with iQ supermix, using primers and a probe specific to the IL-1 β gene. Eight replicates at each template concentration were amplified along with no-template controls on the MyiQ real-time system. Standard curve had $r = 0.999$, slope = -3.378 , efficiency = 97.7%.



iTaq SYBR Green supermix with ROX produces highly uniform results on the ABI PRISM 7000 sequence detection system. The Bio-Rad supermix was compared to supplier A's ROX-containing supermix by amplifying 10^2 copies of the β -actin gene from a plasmid template (48 replicates per mix). The Bio-Rad supermix generated more uniform results with an earlier average C_t of 24.4 (SD = 0.229) than supplier A's ROX-containing supermix (average C_t = 28.0, SD = 0.433). All reactions were performed on the same 96-well plate.



Linearity of four-target detection using the iQ5 real-time PCR detection system. A series of 10-fold dilutions of human genomic DNA (500 ng–50 μ g per 50 μ l reaction) was amplified using iQ multiplex powermix. Targets were α -tubulin (detected with a FAM-labeled probe; efficiency = 96.4%, $r^2 = 0.998$); GAPDH (HEX-labeled probe; efficiency = 94.9%, $r^2 = 0.999$); IL-1 β (Texas Red-labeled probe; efficiency = 101.8%, $r^2 = 0.999$); and factor VIII (Cy5-labeled probe; efficiency = 101.6%, $r^2 = 0.997$).

iProof High-Fidelity DNA Polymerase

iProof high-fidelity DNA polymerase consists of a unique *Pyrococcus*-like proofreading enzyme fused to a dsDNA binding protein, Sso7d. This novel technology results in a thermostable polymerase capable of amplifying long products from a variety of DNA templates while providing the highest fidelity of any available polymerase (52-fold more accurate than *Taq*). iProof polymerase is available in three convenient formulations: a stand-alone enzyme, an easy-to-use master mix, and a PCR kit complete with controls.

- Fidelity — novel proofreading enzyme is the most accurate thermostable polymerase (52-fold more accurate than *Taq*)
- Speed — increased processivity dramatically reduces extension steps (15–30 sec/kb) and overall reaction times
- Length — large fragments (up to 37 kb) are amplified in less time and with less enzyme (0.25–1.0 U/reaction)

iTaq DNA Polymerase

iTaq DNA polymerase is a hot-start DNA polymerase suitable for many PCR applications. The iTaq DNA polymerase kit contains enough PCR reagents for up to 200 x 50 µl reactions using 1.25 U of iTaq DNA polymerase per reaction. The hot-start attribute is mediated through a highly specific antibody. The enzyme is activated after an initial 3 minute denaturation step at 95°C to ensure ease of use and high specificity.

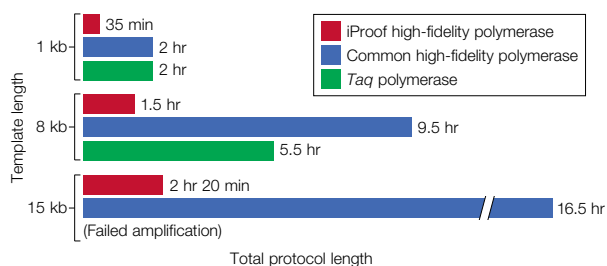
- Antibody-mediated hot-start DNA polymerase for sensitive and specific amplification, with simple 3 minute activation
- Performance tested over a broad range of genomic and plasmid DNA targets
- Qualified for use in both conventional and real-time PCR

dNTP Mix

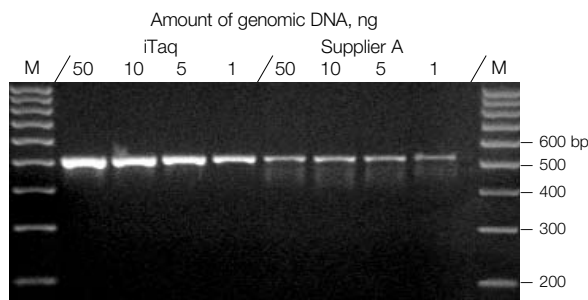
Bio-Rad's dNTP mix is formulated for optimal performance in real-time PCR applications and is also qualified for use in conventional PCR applications. This robust dNTP solution withstands multiple rounds of freeze-thawing and temperature cycling, ensuring consistent and high-yield amplification performance.

ROX Passive Reference Dye

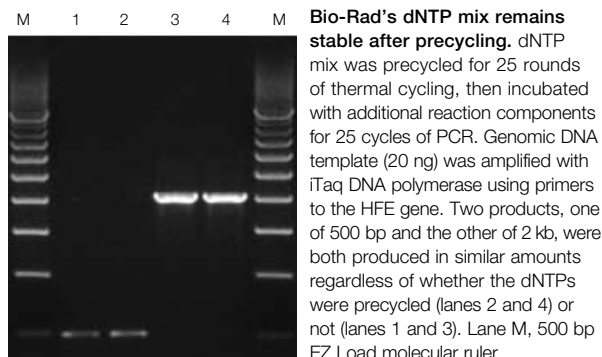
Many real-time thermal cyclers require an internal reference dye for fluorescent signal normalization and correction of well-to-well optical variations. ROX passive reference dye allows seamless integration of non-ROX-containing PCR reagents on all ROX-dependent real-time instrument systems. An internal reference is not required for use with any Bio-Rad real-time detection system.



For long 1–15 kb targets, use of iProof polymerase reduces run times 3- to 4-fold. Targets of 1, 8, or 15 kb were amplified using three different polymerases. A two-step PCR protocol was used with iProof polymerase; three-step protocols using the shortest recommended extension times were used with other polymerases. Because iProof polymerase requires an annealing temperature 5–8°C above typical annealing temperatures, two-step protocols often can be run without redesigning primers.



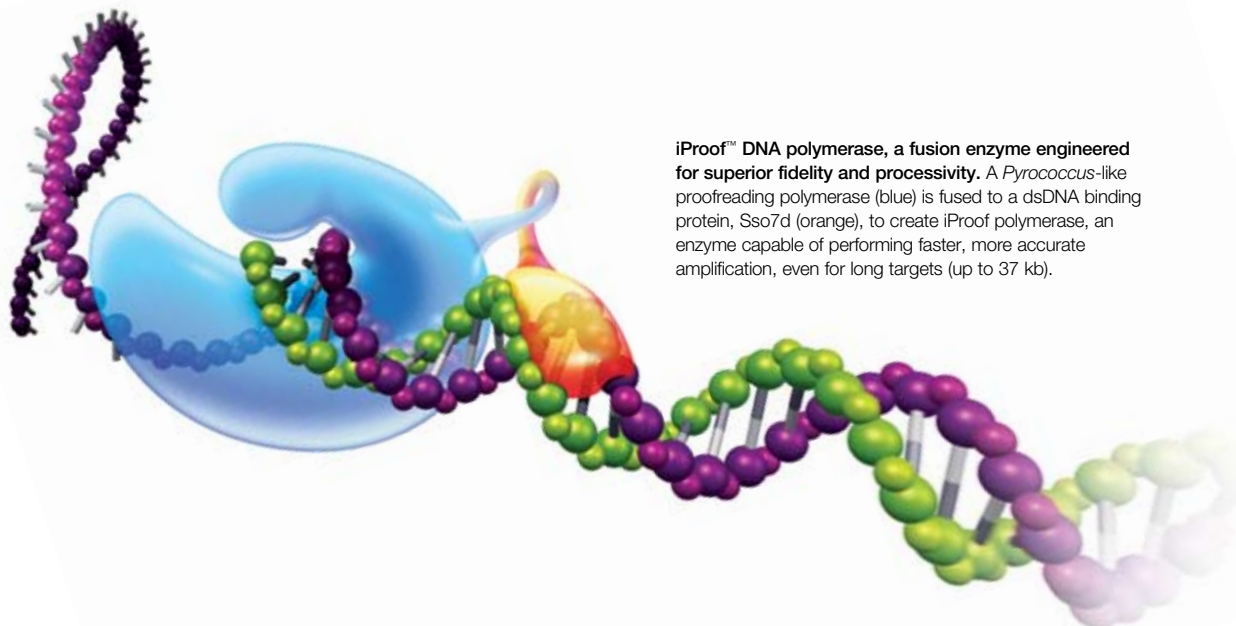
iTaq DNA polymerase performs better than supplier A, even at low target concentrations. Different amounts (indicated above) of a 510 bp target in human genomic DNA were amplified with iTaq DNA polymerase or another supplier's hot-start DNA polymerase according to manufacturers' recommendations. Lane M, EZ Load™ 100 bp molecular ruler.



Bio-Rad's dNTP mix remains stable after precycling. dNTP mix was precycled for 25 rounds of thermal cycling, then incubated with additional reaction components for 25 cycles of PCR. Genomic DNA template (20 ng) was amplified with iTaq DNA polymerase using primers to the HFE gene. Two products, one of 500 bp and the other of 2 kb, were both produced in similar amounts regardless of whether the dNTPs were precycled (lanes 2 and 4) or not (lanes 1 and 3). Lane M, 500 bp EZ Load molecular ruler.

Our Reagents Can Improve Your Reverse Transcription and PCR

Bio-Rad's reagents help you achieve success in all your nucleic acid amplification applications. These reagents are specially formulated for use in reverse transcription, as well as both conventional and real-time PCR applications, with minimal optimization. All reagents are suitable for use in our entire line of thermal cyclers and real-time PCR detection systems. Additionally, all reagents demonstrate high performance over a wide dynamic range for input RNA, cDNA, genomic DNA, and plasmid DNA.



Amplification Reagents Selection Guide

Application	Reagents
High-Fidelity, Long, or Fast PCR	iProof high-fidelity DNA polymerase
Hot-Start PCR or Quantitative PCR	iTaq™ DNA polymerase, dNTP mix
Quantitative PCR	
SYBR Green I-based detection on non-ROX-dependent thermal cyclers	iQ™ SYBR® Green supermix
Probe-based detection on non-ROX-dependent thermal cyclers	iQ™ supermix
SYBR Green I-based detection on ROX-dependent thermal cyclers	iTaq™ SYBR® Green supermix with ROX or iTaq fast SYBR Green supermix with ROX
Probe-based detection on ROX-dependent thermal cyclers	iTaq supermix with ROX or iTaq fast supermix with ROX
Probe-based multi-target detection	iQ multiplex powermix
Two-Step RT-PCR	
cDNA synthesized using a blend of random and oligo(dT) primers	iScript™ cDNA synthesis kit; iTaq DNA polymerase; dNTP mix
cDNA synthesized using random or gene-specific primers	iScript™ Select cDNA synthesis kit; iTaq DNA polymerase; dNTP mix
Two-Step Quantitative RT-PCR	
Non-ROX-dependent thermal cyclers	iScript Select cDNA synthesis kit or iScript cDNA synthesis kit; iQ supermix, iQ SYBR Green supermix, or iQ multiplex powermix
ROX-dependent thermal cyclers	iScript select cDNA synthesis kit or iScript cDNA synthesis kit; iTaq supermix with ROX, iTaq SYBR Green supermix with ROX, iQ multiplex powermix, or ROX passive reference dye
One-Step Quantitative RT-PCR	
SYBR Green I-based detection	iScript™ one-step RT-PCR kit with SYBR® Green
Probe-based detection	iScript one-step RT-PCR kit for probes

Ordering Information

Catalog #	Description	Catalog #	Description
Products for Reverse Transcription		Real-Time PCR Supermixes	
170-8890	iScript cDNA Synthesis Kit , 25 x 20 µl reactions, includes 5x iScript reaction mix, iScript enzyme, nuclease-free water	172-5848	iQ Multiplex Powermix , 50 x 50 µl reactions, 2x mix contains dNTPs, 11 mM MgCl ₂ , iTaq DNA polymerase, stabilizers
170-8891	iScript cDNA Synthesis Kit , 100 x 20 µl reactions	172-5849	iQ Multiplex Powermix , 200 x 50 µl reactions
170-8896	iScript Select cDNA Synthesis Kit , 25 x 20 µl reactions, includes 5x iScript select reaction mix, iScript reverse transcriptase, oligo(dT) mix, random primer mix, gene-specific primer (GSP) enhancer solution, nuclease-free water	172-5850	iTaq SYBR Green Supermix With ROX , 200 x 50 µl reactions, 2x mix contains 0.4 mM each of dATP, dCTP, dGTP, and dTTP, 50 U/ml iTaq DNA polymerase, 6 mM Mg ²⁺ , SYBR Green I, ROX reference dye, stabilizers
170-8897	iScript Select cDNA Synthesis Kit , 100 x 20 µl reactions	172-5851	iTaq SYBR Green Supermix With ROX , 500 x 50 µl reactions
170-8892	iScript One-Step RT-PCR Kit With SYBR Green , 50 reactions	172-5854	iTaq Supermix With ROX , 200 x 50 µl reactions, 2x mix contains 0.4 mM each of dATP, dCTP, dGTP, and dTTP, 50 U/ml iTaq DNA polymerase, 10 mM Mg ²⁺ , ROX reference dye, stabilizers
170-8893	iScript One-Step RT-PCR Kit With SYBR Green , 200 reactions	172-5855	iTaq Supermix With ROX , 500 x 50 µl reactions
170-8894	iScript One-Step RT-PCR Kit for Probes , 50 reactions	170-8860	iQ Supermix , 100 x 50 µl reactions, 2x mix contains 100 mM KCl, 40 mM Tris-HCl, pH 8.4, 0.4 mM each dNTP (dATP, dCTP, dGTP, dTTP), 50 U/ml iTaq DNA polymerase, 6 mM MgCl ₂ , stabilizers
170-8895	iScript One-Step RT-PCR Kit for Probes , 200 reactions	170-8862	iQ Supermix , 500 x 50 µl reactions
Core Reagents		170-8880	iQ SYBR Green Supermix , 100 x 50 µl reactions, 2x mix contains 100 mM KCl, 40 mM Tris-HCl, pH 8.4, 0.4 mM each dNTP (dATP, dCTP, dGTP, dTTP), 50 U/ml iTaq DNA polymerase, 6 mM MgCl ₂ , SYBR Green I, 20 nM fluorescein, stabilizers
170-8870	iTaq DNA Polymerase , 5 U/µl, includes 250 U polymerase, 1.25 ml of 10x PCR buffer, 1.25 ml of 50 mM MgCl ₂ solution	170-8882	iQ SYBR Green Supermix , 500 x 50 µl reactions
170-8875	iTaq DNA Polymerase , 5 U/µl, includes 5,000 U polymerase, 25 ml 10x PCR buffer, of 25 ml of 50 mM MgCl ₂ solution	172-5100	iTaq Fast SYBR Green Supermix With ROX , 200 x 20 µl reactions, 2x mix contains dNTPs, iTaq DNA polymerase, 6 mM Mg ²⁺ , SYBR Green I, ROX passive reference dye, stabilizers
172-5300	iProof High-Fidelity DNA Polymerase , 2 U/µl, 20 U enzyme, includes 5x reaction buffers, MgCl ₂ solution, DMSO	172-5105	iTaq Fast Supermix With ROX , 200 x 20 µl reactions, 2x mix contains dNTPs, iTaq DNA polymerase, 6 mM Mg ²⁺ , ROX passive reference dye, stabilizers
172-5301	iProof High-Fidelity DNA Polymerase , 2 U/µl, 100 U	172-5106	iTaq Fast Supermix With ROX , 500 x 20 µl reactions
172-5302	iProof High-Fidelity DNA Polymerase , 2 U/µl, 500 U		
172-5310	iProof HF Master Mix , 100 x 50 µl reactions, includes 2x master mix, DMSO (for highest fidelity with most templates)		
172-5311	iProof HF Master Mix , 500 x 50 µl reactions		
172-5320	iProof GC Master Mix , 100 x 50 µl reactions, includes 2x master mix, DMSO (for GC-rich templates)		
172-5321	iProof GC Master Mix , 500 x 50 µl reactions		
172-5330	iProof High-Fidelity PCR Kit , 2 U/µl, 50 U, includes 5x reaction buffers, MgCl ₂ , DMSO, dNTPs, DNA, 1.3 and 10 kb primers, DNA standard		
172-5331	iProof High-Fidelity PCR Kit , 2 U/µl, 200 U		
172-5391	5x iProof HF Buffer		
172-5392	5x iProof GC Buffer		
172-5393	5x iProof HPLC HF Buffer , detergent-free		
172-5394	5x iProof HPLC GC Buffer , detergent-free		
170-8872	MgCl₂ Solution , 50 mM, 1.25 ml		
172-5858	ROX Passive Reference Dye , 0.5 ml		
170-8874	dNTP Mix , 200 µl premixed solution, contains 10 mM each dNTP (dATP, dCTP, dGTP, dTTP)		

Bio-Rad's real-time thermal cyclers are licensed real-time thermal cyclers under Applera's United States Patent No. 6,814,934 B1 for use in research and for all other fields except the fields of human diagnostics and veterinary diagnostics.

Practice of the patented 5' Nuclease Process requires a license from Applied Biosystems. The purchase of these products includes an immunity from suit under patents specified in the product insert to use only the amount purchased for the purchaser's own internal research when used with the separate purchase of Licensed Probe. No other patent rights are conveyed expressly, by implication, or by estoppel. Further information on purchasing licenses may be obtained from the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

ABI PRISM is a trademark of Applied Biosystems. Cy is a trademark of GE Healthcare. SYBR and Texas Red are trademarks of Invitrogen Corporation. Bio-Rad Laboratories, Inc. is licensed by Invitrogen Corporation to sell reagents containing SYBR Green I for use in real-time PCR, for research purposes only.

BIO-RAD

**Bio-Rad
Laboratories, Inc.**

Life Science
Group

Web site www.bio-rad.com USA 800 4BIORAD Australia 61 02 9914 2800 Austria 01 877 89 01 Belgium 09 385 55 11 Brazil 55 21 3237 9400 Canada 905 364 3435 China 86 21 6426 0808 Czech Republic 420 241 430 532 Denmark 44 52 10 00 Finland 09 804 22 00 France 01 47 95 69 65 Germany 089 318 84 0 Greece 30 210 777 4396 Hong Kong 852 2789 3300 Hungary 36 1 455 8800 India 91 124 4029300 Israel 03 963 6050 Italy 39 02 216091 Japan 03 6361 7000 Korea 82 2 3473 4460 Mexico 52 555 488 7670 The Netherlands 0318 540666 New Zealand 0508 805 500 Norway 23 38 41 30 Poland 48 22 331 99 99 Portugal 351 21 472 7700 Russia 7 495 721 14 04 Singapore 65 6415 3188 South Africa 27 861 246 723 Spain 34 91 590 5200 Sweden 08 555 12700 Switzerland 061 717 95 55 Taiwan 886 2 2578 7189 United Kingdom 020 8328 2000