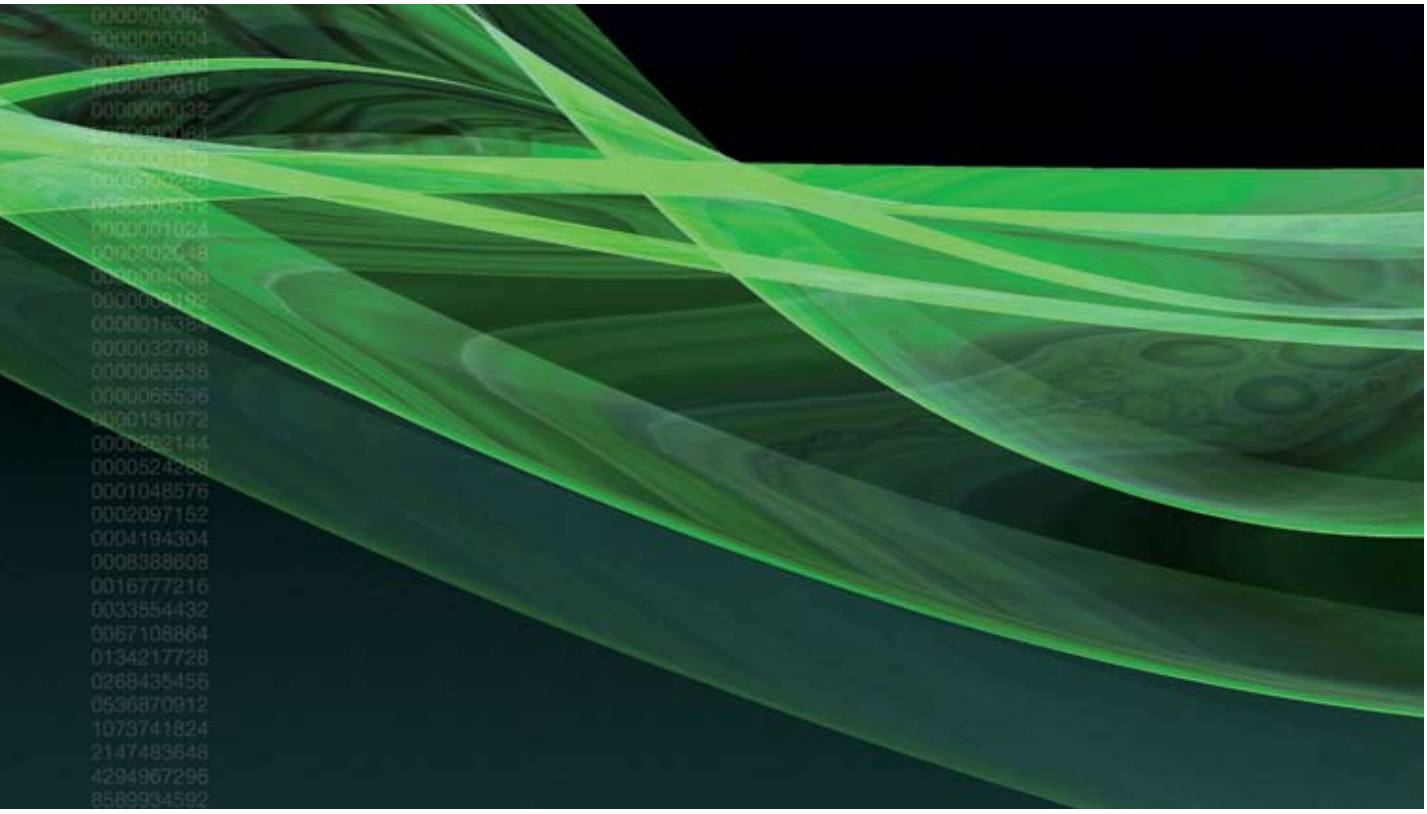


# Reagents

For Reverse Transcription, PCR, and Real-Time PCR

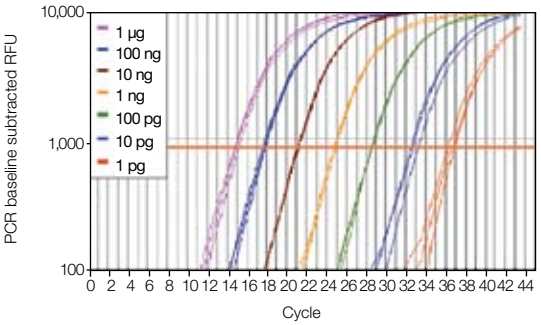
BIO-RAD

## 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



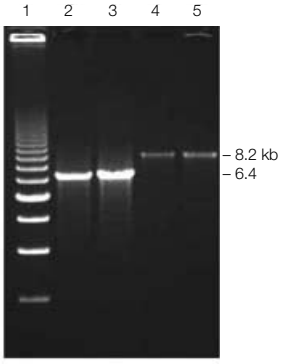

**The iScript cDNA synthesis kit performs across a broad range of concentrations.** Input RNA was reverse transcribed, amplified using iQ SYBR Green supermix, and detected on the iCycler iQ® real-time PCR detection system. Bio-Rad's iScript cDNA synthesis kit delivered a dynamic range of 6 orders of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

## 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



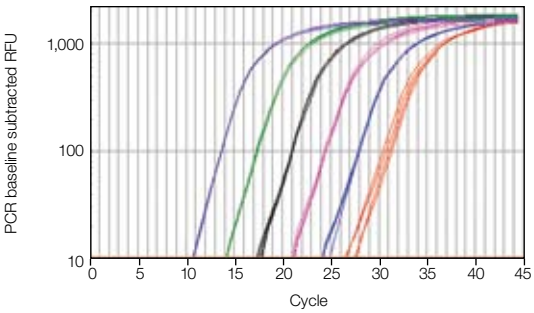

**The iScript Select cDNA synthesis kit facilitates synthesis of cDNA longer than 6 kb.** First-strand cDNA was produced from 1 µg total RNA using the iScript Select cDNA synthesis kit and the provided oligo(dT) primer mix. A 2 µl aliquot of the 20 µl cDNA synthesis reaction was subjected to 35 PCR cycles 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.

## 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



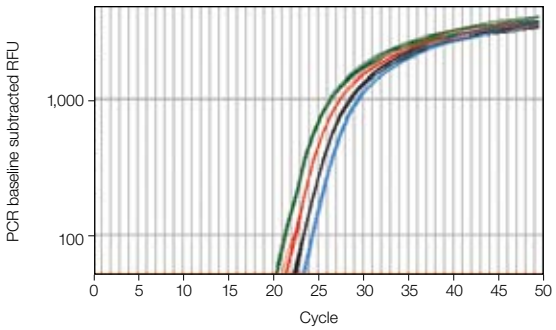

**The iScript one-step RT-PCR kit with SYBR Green provides high sensitivity across a broad range of concentrations.** One-step RT-PCR reactions were performed in triplicate, along with no-template controls, using GAPDH primers and 100 ng to 100 fg of total HeLa RNA. Reactions were carried out on the 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



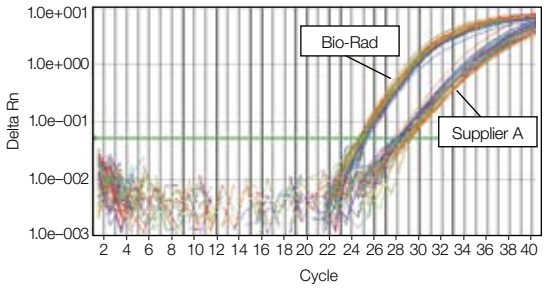

**An accurate one-cycle spacing in $C_T$ is precisely maintained in a series of 2-fold dilutions.** Human genomic DNA was amplified with iQ supermix, using primers and a probe specific to the IL-1β gene. Eight replicates at each template concentration were amplified along with no-template controls on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

## iTaq 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



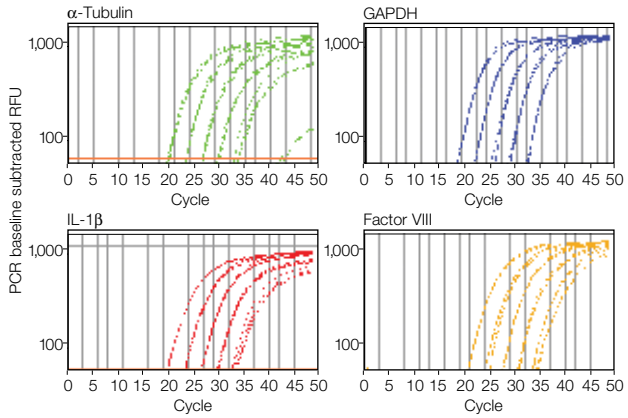

**iTaq SYBR Green supermix with ROX produces highly uniform results on the ABI PRISM 7000 sequence detection system.** The Bio-Rad supermix was compared to supplier A's ROX-containing supermix by amplifying $10^2$ copies of the β-actin gene from a plasmid template (48 replicates per mix). The Bio-Rad 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.

## 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

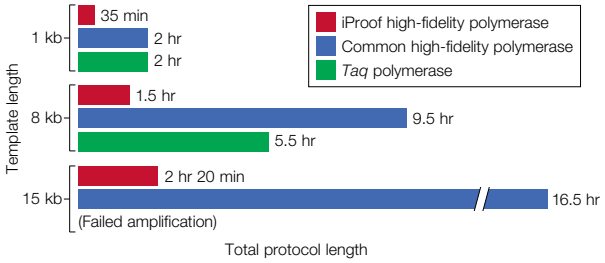



**Linearity of four-target detection using the iQ5 real-time PCR detection system.** A series of 10-fold dilutions of human genomic DNA (500 ng–50 pg per 50 µl reaction) was amplified using iQ multiplex powermix. Targets were α-tubulin (detected with a FAM-labeled probe; efficiency = 96.4%, $r^2$ = 0.998);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

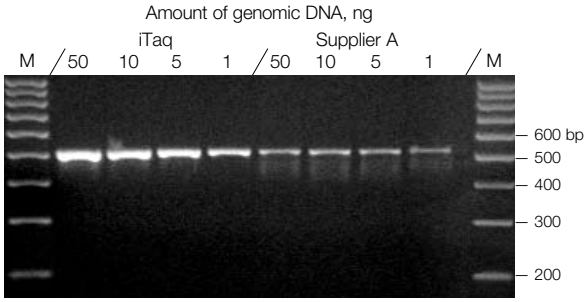



**For long 1–15 kb targets, use of iProof polymerase reduces run times 3- to 4-fold.** Targets of 1, 8, or 15 kb were amplified using three different polymerases. A two-step PCR protocol was used with iProof polymerase; three-step protocols using the shortest recommended extension times were used with 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

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.

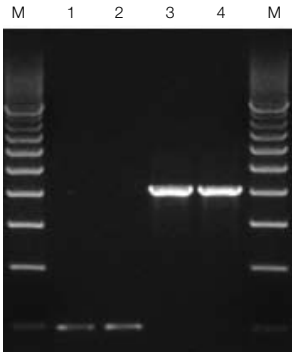
- Antibody-mediated hot-start DNA polymerase for sensitive and specific amplification, with simple 3 minute activation
- Performance tested over a broad range of genomic and plasmid DNA targets
- Qualified for use in both conventional and real-time PCR




**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.

## 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.



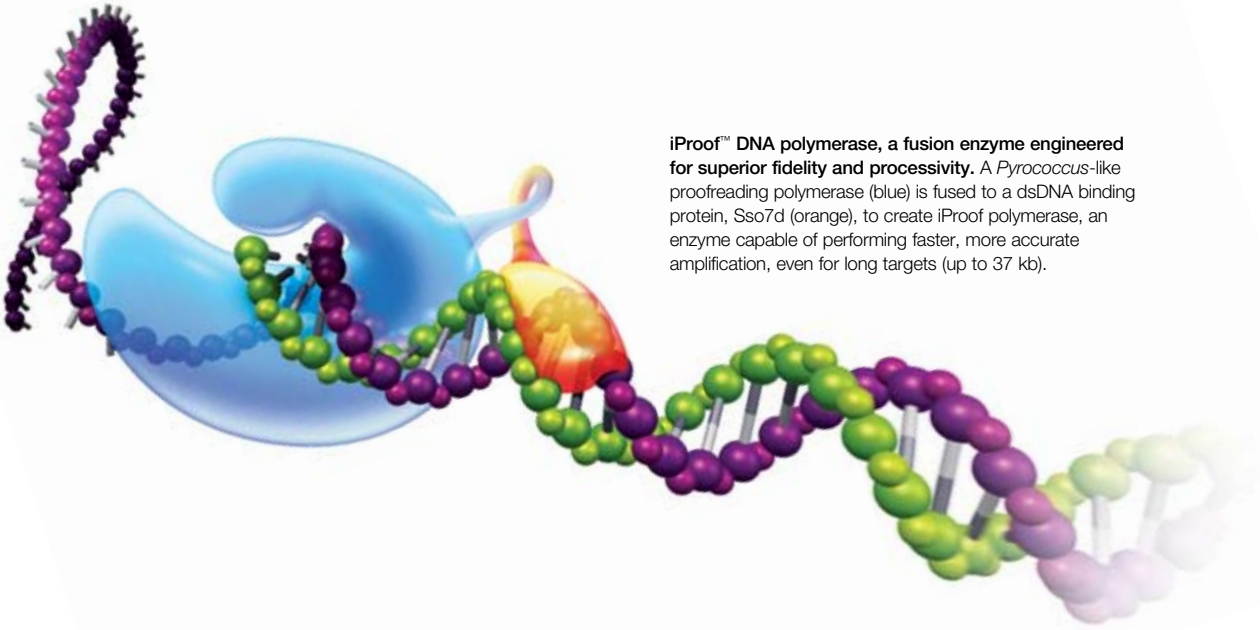

**Bio-Rad's dNTP mix remains stable after precycling.** dNTP mix was precycled for 25 rounds of thermal cycling, then incubated with additional reaction components for 25 cycles of PCR. Genomic DNA template (20 ng) was amplified with iTaq DNA polymerase using primers to the HFE gene. Two products, one 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.

## 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.

# 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.



**iProof™ DNA polymerase, a fusion enzyme engineered for superior fidelity and processivity.** A *Pyrococcus*-like proofreading polymerase (blue) is fused to a dsDNA binding protein, Sso7d (orange), to create iProof polymerase, an enzyme capable of performing faster, more accurate amplification, even for long targets (up to 37 kb).

## 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 |
|---|---|
| **Products for Reverse Transcription** | |
| 170-8890 | **iScript cDNA Synthesis Kit**, 25 x 20 µl reactions, includes 5x iScript reaction mix, iScript enzyme, nuclease-free water |
| 170-8891 | **iScript cDNA Synthesis Kit**, 100 x 20 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 |
| 170-8897 | **iScript Select cDNA Synthesis Kit**, 100 x 20 µl reactions |
| 170-8892 | **iScript One-Step RT-PCR Kit With SYBR Green**, 50 reactions |
| 170-8893 | **iScript One-Step RT-PCR Kit With SYBR Green**, 200 reactions |
| 170-8894 | **iScript One-Step RT-PCR Kit for Probes**, 50 reactions |
| 170-8895 | **iScript One-Step RT-PCR Kit for Probes**, 200 reactions |
| **Core Reagents** | |
| 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 |
| 170-8875 | **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 |
| 172-5300 | **iProof High-Fidelity DNA Polymerase**, 2 U/µl, 20 U enzyme, includes 5x reaction buffers, $MgCl_2$ solution, DMSO |
| 172-5301 | **iProof High-Fidelity DNA Polymerase**, 2 U/µl, 100 U |
| 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, λDNA, 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 | **$MgCl_2$ 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) |

| Catalog # | Description |
|---|---|
| **Real-Time PCR Supermixes** | |
| 172-5848 | **iQ Multiplex Powermix**, 50 x 50 µl reactions, 2x mix contains dNTPs, 11 mM $MgCl_2$, iTaq DNA polymerase, stabilizers |
| 172-5849 | **iQ Multiplex Powermix**, 200 x 50 µl reactions |
| 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 |
| 172-5851 | **iTaq SYBR Green Supermix With ROX**, 500 x 50 µl 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 |
| 172-5855 | **iTaq Supermix With ROX**, 500 x 50 µl 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 |
| 170-8862 | **iQ Supermix**, 500 x 50 µl reactions |
| 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 |
| 170-8882 | **iQ SYBR Green Supermix**, 500 x 50 µl reactions |
| 172-5100 | **iTaq Fast SYBR Green Supermix With ROX**, 200 x 20 µl reactions, 2x mix contains dNTPs, iTaq DNA polymerase, 6 mM Mg2+, SYBR Green I, ROX passive reference dye, stabilizers |
| 172-5101 | **iTaq Fast SYBR Green Supermix With ROX**, 500 x 20 µl reactions |
| 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 |
| 172-5106 | **iTaq Fast Supermix With ROX**, 500 x 20 µl reactions |



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