PCR AND GENE EXPRESSION WORKFLOW

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1 CELL LYSIS

SingleShot™ Cell Lysis RT-qPCR Kits provide a complete and fast solution for generation of lysates from cell cultures. These lysates are optimized for downstream one- or two-step quantitative PCR (qPCR) reactions, and do not require an RNA purification step.

- Ready-to-use cell lysate from 10 to 100,000 cells in 20 minutes
- Simple protocol for automated, high-throughput reverse transcription qPCR (RT-qPCR) experiments
- One- or two-step SYBR® Green or probes kits
SingleShot™ Cell Lysis RT-qPCR Kits

All SingleShot Cell Lysis RT-qPCR Kits feature:
- Complete removal of genomic DNA (gDNA) without the need for purification
- Preservation of RNA integrity by our potent blend of RNase inhibitors
- No loss of rare transcripts from column purification
- Optimal accuracy and high sensitivity of qPCR data
- Validated with PrimePCR™ Assays and Panels and workflows for SsoAdvanced™ PreAmp Supermix and PrimePCR PreAmp Assays
- Comparable results to those obtained when using purified RNA

SingleShot Kits are available in the following formats:
- SingleShot Cell Lysis Kits
- SingleShot Cell Lysis Two-Step RT-qPCR Kits
- SingleShot Cell Lysis One-Step RT-qPCR Kits

### SingleShot workflow.

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Seed cells</td>
</tr>
<tr>
<td>2</td>
<td>Add lysis buffer containing proteinase K + DNase</td>
</tr>
<tr>
<td>3</td>
<td>Incubate 10 min at room temperature</td>
</tr>
<tr>
<td>4</td>
<td>Heat 10 min</td>
</tr>
<tr>
<td>5</td>
<td>Transfer</td>
</tr>
<tr>
<td>6</td>
<td>PCR plate</td>
</tr>
</tbody>
</table>

### SingleShot workflow.

<table>
<thead>
<tr>
<th>SingleShot Kit</th>
<th>Features</th>
<th>Product Options</th>
</tr>
</thead>
</table>
| Cell Lysis Kits | - Cell lysis for RT-qPCR  
- RT-qPCR reagents sold separately  
- No RNA purification needed | 100 reactions, catalog #172-5080  
500 reactions, catalog #172-5081 |
| Cell Lysis Two-Step RT-qPCR Kits | - Cell lysis, reverse transcription, and qPCR  
- iScript™ Advanced cDNA Synthesis Kit for RT-qPCR included  
- SsoAdvanced Universal Supermix included  
- No RNA purification needed | SYBR® Green, 100 reactions, catalog #172-5085  
Probes, 100 reactions, catalog #172-5090 |
| Cell Lysis One-Step RT-qPCR Kits | - Cell lysis, reverse transcription, and qPCR  
- iTaq™ Universal One-Step Kit included  
- No RNA purification needed | SYBR® Green, 100 reactions, catalog #172-5095  
Probes, 100 reactions, catalog #172-5070 |
One-Step and Two-Step SYBR® Green or Probes Formats

**SingleShot™ Cell Lysis Kits**
- Kits rapidly generate cell lysates that are optimized for RT-qPCR analysis without RNA purification
- Superior gDNA removal while preserving RNA integrity
- Minimal hands-on protocol provides a high-throughput solution

**Reproducibility of Cell Lysates**

![Pearson r = 0.99]

The SingleShot™ SYBR® Green Kit demonstrates high reproducibility between technical lysate replicates. Two technical lysate replicates of four neuroblastoma cell lines (SH-EP, SK-N-AS, NGP, and IMR-32) were examined using ten SYBR® Green qPCR assays to evaluate the reproducibility of the SingleShot™ SYBR® Green Kit. A Pearson correlation of 0.99 was observed, thus demonstrating highly reproducible data between lysis reactions. Cq, quantification cycle.

Data used with permission from Gert Van Peer, Pieter Mestdagh, and Jo Vandesompele, Center for Medical Genetics, Ghent University, Ghent, Belgium.

**SingleShot Cell Lysis Two-Step RT-qPCR Kits**
- Kits yield high-performance RT-qPCR data directly from cell culture lysates in less than 2 hours after cell lysis
- Available with SYBR® Green or probe chemistry
- SingleShot RNA Control is included to ensure optimal input cells and lysates

**PCR Efficiency across Expression Levels**

![Input cells (log)]

SingleShot Probes Kit maintains high PCR efficiencies across all expression levels. Accurate gene expression studies require that PCR efficiencies be within 90–110%, per the minimum information for publication of quantitative real-time PCR experiments (MIQE) guidelines. Using the SingleShot Probes Kit, three targets of varying expression levels were analyzed using input cell numbers ranging from $10^5$ to 10 HeLa cells. Greater than 98% PCR efficiencies were maintained regardless of the expression levels ($TBP$, 99.6%; $HPRT$, 98.6%; $B2M$, 99.2%) and sensitivity down to 10 cells was observed. The SingleShot RNA Control was used to monitor PCR inhibition. As the control quantification cycle (Cq) values remained constant, no inhibition was noted.
One-Step and Two-Step SYBR® Green or Probes Formats

SingleShot™ Cell Lysis One-Step RT-qPCR Kits
- Within 1.5 hr after cell lysis, yields high-performance RT-qPCR data directly from cell culture lysates
- Cell lysate from 10 to 100,000 cells ready in 20 min
- Linear dynamic range across genes
- Available with SYBR® Green or probe chemistry
- SingleShot RNA Control is included to ensure optimal input cells and lysates

For more information, download or request bulletins 6572 and 6604.
Bio-Rad provides two distinct technologies for isolating total RNA. Silica membrane–based kits, available in 96-well plate and spin column formats, and PureZOL™ RNA Isolation Reagent, which is ideal for scaling up isolation protocols.

- Kits are designed and formulated to assist in the isolation of highly pure and intact RNA from different starting materials
- RNA is compatible with a variety of downstream applications
  - Real-time qPCR
  - Northern blotting
  - Microarray analysis
  - cDNA library construction
- DNase treatment ensures gDNA removal
Aurum™ Sample Preparation Kits and PureZOL™ RNA Isolation Reagent

Aurum Total RNA Kits
Aurum Total RNA Kits are a family of isolation kits that provide a high yield of intact RNA from a wide range of starting materials, including cultured cells, bacteria, and yeast, as well as plant and animal tissues.

- PCR-ready RNA in less than 60 min
- RNase-free reagents and plastic consumables ensure the integrity of isolated RNA
- Kit includes DNase I provided for removal of gDNA contamination
- Easy-to-use spin or vacuum protocol

Aurum Total RNA Isolation Products come in:
- Aurum Total RNA Fatty and Fibrous Tissue Kit
- Aurum Total RNA Mini Kit
- Aurum Total RNA 96 Kit

PureZOL™ RNA Isolation Reagent

- Single-solution format permits recovery of RNA from small quantities of tissues or cells, making it ideally suited for gene expression studies
- Efficient RNA purification from cultured cells, yeast, viruses, and bacteria, as well as plant and animal tissues
- PureZOL efficiently lyses cells and tissues, deproteinates RNA, and inactivates endogenous nucleases in a single step
- Scalable starting sample amount
- Convenient isolation of RNA, DNA, and protein from the same sample

For more information, download or request bulletins 2919, 2920, and 5282.
## RNA Isolation Kits Selection Guide

### Aurum™ Total RNA Kits

<table>
<thead>
<tr>
<th>Description</th>
<th>Mini Column</th>
<th>Fatty and Fibrous Tissue</th>
<th>96-Well Plate</th>
<th>PureZOL™ RNA Isolation Reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>Guanidine isothiocyanate and ß-mercaptoethanol efficiently lyse samples and quickly inactivate RNases</td>
<td>Kit combines PureZOL, which effectively lyses tissues and cells with the speed of silica membrane technology in a spin column format to yield high-quality, intact RNA</td>
<td>Kit is composed of guanidine isothiocyanate and ß-mercaptoethanol for efficient lysis and quick RNase inactivation, followed by purification in a 96-well plate</td>
<td>PureZOL efficiently lyses cells and tissues, deproteinates RNA, and inactivates endogenous nucleases in a single step</td>
</tr>
<tr>
<td>Format</td>
<td>Mini column</td>
<td>Mini column</td>
<td>96-well plate</td>
<td>Single solution organic extraction</td>
</tr>
<tr>
<td>Maximum starting material amounts</td>
<td>Cultured cells: 2 x 10⁶, 1 x 10⁷, 1 x 10⁶, 1 x 10⁷</td>
<td>Bacterial cells: 2.4 x 10⁹, 2.4 x 10⁹, 8 x 10⁸, 2.4 x 10⁹</td>
<td>Yeast cells: 3 x 10⁷, 3 x 10⁷, 2 x 10⁷, 3 x 10⁷</td>
<td>Hard animal tissue: 20 mg, 100 mg, 100 mg, 100 mg</td>
</tr>
<tr>
<td></td>
<td>Soft to moderately hard animal tissue: 40 mg, 100 mg</td>
<td>Plant tissue: 40 mg, 100 mg</td>
<td>Isolation method: Silica membrane, Lysis with PureZOL reagent, purification on silica membrane</td>
<td>Isolation method: Single solution organic extraction</td>
</tr>
<tr>
<td></td>
<td>Number of preps: 50 mini preps, 50 mini preps</td>
<td>2 x 96-well plate</td>
<td>Number of washes: 3, 3</td>
<td>Number of washes: 3, 3</td>
</tr>
<tr>
<td></td>
<td>DNase I included*: Yes, Yes</td>
<td>DNase I digest time: 15 min (animal tissue, 25 min), 15 min</td>
<td>Total preparation time**: &lt;50–80 min (with DNase I digest), &lt;50–80 min (with DNase I digest)</td>
<td>Total preparation time**: &lt;50–80 min (with DNase I digest), &lt;50–80 min (with DNase I digest)</td>
</tr>
<tr>
<td></td>
<td>Binding capacity: &gt;100 µg, &gt;100 µg</td>
<td>Elution volume: 2 x 40 µl, 2 x 40 µl</td>
<td>Binding capacity: &gt;100 µg, &gt;100 µg</td>
<td>Elution volume: 80 µl, 80 µl</td>
</tr>
</tbody>
</table>

* Removal not required.  
** Total preparation time will vary depending on the tissue or cell type and on which format is used (vacuum or spin).
### Ordering Information

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>732-6830</td>
<td>Aurum Total RNA Fatty and Fibrous Tissue Kit</td>
</tr>
<tr>
<td>732-6870*</td>
<td>Aurum Total RNA Fatty and Fibrous Tissue Module</td>
</tr>
<tr>
<td>732-6820</td>
<td>Aurum Total RNA Mini Kit</td>
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<tr>
<td>732-6800</td>
<td>Aurum Total RNA 96 Kit</td>
</tr>
<tr>
<td>732-6880</td>
<td>PureZOL RNA Isolation Reagent, 50 ml</td>
</tr>
<tr>
<td>732-6890</td>
<td>PureZOL RNA Isolation Reagent, 100 ml</td>
</tr>
</tbody>
</table>

* Not provided with PureZOL RNA Isolation Reagent (see catalog #732-6890 or #732-6880 to order separately).
Bio-Rad’s reverse transcription kits, in one- or two-tube formats, provide easy setup and high reproducibility for first-strand cDNA synthesis. Bio-Rad’s reverse transcription reagents cover a range of priming strategies, including primer blends.

- Formulated for efficient reverse transcription across a broad linear dynamic range
- Potent RNase A inhibitors protect RNA during setup and reverse transcription
- Flexible input RNA capacity to suit different experimental needs
- Optimized for gene expression analysis using real-time qPCR
# iScript™ Kit Selector

<table>
<thead>
<tr>
<th>Reduce Pipetting Variability</th>
<th>Maximize Data from Single 20 μl Reaction</th>
<th>Fast and Easy to Use</th>
<th>Select My Own Primers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Product</strong></td>
<td><strong>Maximize Data from Single 20 μl Reaction</strong></td>
<td><strong>Fast and Easy to Use</strong></td>
<td><strong>Select My Own Primers</strong></td>
</tr>
<tr>
<td>iScript Reverse Transcription Supermix for RT-qPCR (one-tube format)</td>
<td>iScript Advanced cDNA Synthesis Kit for RT-qPCR (two-tube format)</td>
<td>iScript cDNA Synthesis Kit (two-tube format)</td>
<td>iScript Select cDNA Synthesis Kit (multi-tube format for ultimate flexibility)</td>
</tr>
<tr>
<td><strong>Input</strong></td>
<td><strong>Input</strong></td>
<td><strong>Input</strong></td>
<td><strong>Input</strong></td>
</tr>
<tr>
<td>1 μg–1 pg total RNA</td>
<td>7.5 μg–100 fg total RNA</td>
<td>1 μg–100 fg total RNA</td>
<td>1 μg–1 pg total RNA</td>
</tr>
<tr>
<td><strong>Kit Contents</strong></td>
<td><strong>Kit Contents</strong></td>
<td><strong>Kit Contents</strong></td>
<td><strong>Kit Contents</strong></td>
</tr>
<tr>
<td>■ 5x iScript RT Supermix (dNTPs, oligo[dT], random primers, buffer components, and iScript Reverse Transcriptase)</td>
<td>■ iScript Reverse Transcriptase</td>
<td>■ iScript Reverse Transcriptase</td>
<td>■ iScript Reverse Transcriptase</td>
</tr>
<tr>
<td>■ 5x iScript Advanced Reaction Mix (dNTPs, oligo[dT], random primers, and buffer components)</td>
<td>■ 5x iScript Reaction Mix (dNTPs, oligo[dT], random primers, and buffer components)</td>
<td>■ 5x iScript Reaction Mix (dNTPs, oligo[dT], random primers, and buffer components)</td>
<td>■ 5x iScript Reaction Mix (dNTPs and buffer components)</td>
</tr>
<tr>
<td>■ Oligo(dT), random primers, and gene-specific primer (GSP) enhancer solution (3 vials)</td>
<td>■ Oligo(dT), random primers, and gene-specific primer (GSP) enhancer solution (3 vials)</td>
<td>■ Oligo(dT), random primers, and gene-specific primer (GSP) enhancer solution (3 vials)</td>
<td>■ Oligo(dT), random primers, and gene-specific primer (GSP) enhancer solution (3 vials)</td>
</tr>
<tr>
<td><strong>Results</strong></td>
<td><strong>Results</strong></td>
<td><strong>Results</strong></td>
<td><strong>Results</strong></td>
</tr>
<tr>
<td>cDNA ready in 40 min for qPCR</td>
<td>cDNA ready in 35 min for qPCR</td>
<td>cDNA ready in 40 min for qPCR</td>
<td>cDNA ready in 40–90 min for qPCR</td>
</tr>
</tbody>
</table>
cDNA Synthesis Reagents

iScript™ Performance

How iScript Reagents work:
- RNase H+ Moloney murine leukemia virus (MMLV) reverse transcriptase (preblended with RNase inhibitor) delivers high sensitivity for real-time RT-qPCR and eliminates additional RNase H+ step
- RNase H+ activity of the reverse transcriptase ensures 1:1 RNA:cDNA conversion, resulting in greater sensitivity and more accurate data
- Optimal blend of oligo(dT) and random primers for complete and unbiased 3’ to 5’ coverage
- Potent blend of RNase inhibitors prevents RNA degradation during reaction setup and cDNA synthesis steps

Total RNA (7.5, 2, 1, and 0.1 µg) from HeLa cells was reverse transcribed using the iScript Advanced cDNA Synthesis Kit for RT-qPCR in a 20 µl reaction. One-tenth of the generated cDNA was used as template to amplify the APC gene in a 10 µl qPCR reaction with iTaq™ Universal SYBR® Green Supermix on a CFX384™ Real-Time PCR Detection System. There was no significant difference between the two primer pairs. This demonstrates superior coverage of the 5’ and 3’ regions of target mRNA. RFU, relative fluorescence units.

Superior 5’ to 3’ Coverage

Thermal Cycling Protocol
- 95°C for 3 min
- 45 cycles of 95°C for 10 sec and 60°C for 30 sec
- 5’ end, 91 bp amplicon
- 3’ end, 69 bp amplicon

Broad Dynamic Range

R² = 0.999
Efficiency = 99.7%
Slope = –3.33

iScript Reverse Transcription Supermix for RT-qPCR efficiently reverse transcribes RNA over a broad linear dynamic range for reliable gene expression analysis data. Different amounts of HeLa cell RNA (amounts shown in inset) were reverse transcribed and one-tenth of the resulting cDNA was used as a template to amplify β-actin gene (~90 bp) in 20 µl qPCR reactions with iTaq™ Universal SYBR® Green Supermix. RFU, relative fluorescence units.
cDNA Synthesis Reagents

iScript™ Performance
Bio-Rad’s cDNA Synthesis Kits in one- or two-tube formats provide easy setup and high reproducibility for first-strand cDNA synthesis. The combination of RNase H+ MMLV reverse transcriptase and RNase inhibitor yields reliable data across a wide range of input RNA.

**iScript Reproducibility**

Excellent data reproducibility. PGK-1 mRNA (~160 bp), a gene that encodes a glycolytic enzyme, was quantified using iScript Reverse Transcription Supermix for RT-qPCR, both with 100 ng (■) and 100 pg (▲) of input RNA. For each input RNA, 48 individual RT reactions were performed and one-tenth of the resulting cDNA was used in the qPCR reaction with SsoFast™ Probes Supermix. The gene expression analysis data show excellent reproducibility, both with high and low levels of input target mRNA. The ~10 quantification cycle (Cq) difference for the 1,000-fold dilution of RNA (100 ng–100 pg) demonstrates good reverse transcription efficiencies across different input RNAs. CV, coefficient of variation; RFU, relative fluorescence units; SD, standard deviation.

Unbiased Representation of 5’ and 3’ Regions

Unbiased representation of 5’ and 3’ regions of target genes. Reverse transcription of 100, 10, 1, and 0.1 ng input RNA was performed with iScript Reverse Transcription Supermix for RT-qPCR. Primer pairs were designed at 5’ (■, ~60 bp) and 3’ (▲, ~70 bp) ends of the MAP gene and qPCR was performed with one-tenth of input cDNA using iTaq™ Universal SYBR® Green Supermix. There were no significant differences (<0.5 Cq difference) between the two primer pairs. This demonstrates unbiased representation of 5’ and 3’ regions. RFU, relative fluorescence units.

**Ordering Information**

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Description</th>
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<tbody>
<tr>
<td>172-5037</td>
<td>iScript Advanced cDNA Synthesis Kit for RT-qPCR, 25 x 20 μl reactions</td>
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<tr>
<td>172-5038</td>
<td>iScript Advanced cDNA Synthesis Kit for RT-qPCR, 100 x 20 μl reactions</td>
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<tr>
<td>170-8890</td>
<td>iScript cDNA Synthesis Kit, 25 x 20 μl reactions</td>
</tr>
<tr>
<td>170-8891</td>
<td>iScript cDNA Synthesis Kit, 100 x 20 μl reactions</td>
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<table>
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<th>Catalog #</th>
<th>Description</th>
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<tbody>
<tr>
<td>170-8840</td>
<td>iScript Reverse Transcription Supermix for RT-qPCR, 25 x 20 μl reactions</td>
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<tr>
<td>170-8841</td>
<td>iScript Reverse Transcription Supermix for RT-qPCR, 100 x 20 μl reactions</td>
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<tr>
<td>170-8896</td>
<td>iScript Select cDNA Synthesis Kit, 25 x 20 μl reactions</td>
</tr>
<tr>
<td>170-8897</td>
<td>iScript Select cDNA Synthesis Kit, 100 x 20 μl reactions</td>
</tr>
</tbody>
</table>
Bio-Rad’s PCR and real-time PCR supermixes feature a hot-start DNA polymerase for quick activation and an advanced enzyme and buffer formulation for robust amplification.

- Patented Sso7d fusion enzyme technology delivers higher processivity and inhibitor tolerance
- High performance obtained on any instrument, under any condition
- Antibody-mediated hot-start technology enables instant polymerase activation and superior specificity
- Time to results decreased without compromising qPCR data quality
# Real-Time PCR Reagents Selection Guide

Use this selection guide to choose an appropriate universal supermix for your sample, target, or application.

<table>
<thead>
<tr>
<th>Product</th>
<th>Samples</th>
<th>Targets</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SsoAdvanced™ Universal SYBR® Green Supermix</strong></td>
<td>Blood spot*</td>
<td>AT- and GC-rich regions</td>
<td>Chromatin immunoprecipitation (ChIP)</td>
</tr>
<tr>
<td></td>
<td>Cell lysate*</td>
<td>50–250 bp amplicons</td>
<td>Copy number variation</td>
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<tr>
<td></td>
<td>Crude extraction*</td>
<td>Increased secondary structure</td>
<td>Gene expression analysis</td>
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<tr>
<td></td>
<td>Formalin-fixed, paraffin-embedded (FFPE)</td>
<td>Longer amplicons, &gt;250 bp</td>
<td>Genotyping</td>
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<tr>
<td></td>
<td>Laser capture microdissection (LCM)</td>
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<td>Long noncoding RNA (lncRNA) and microRNA (miRNA) expression analysis</td>
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<tr>
<td></td>
<td>Plant extract</td>
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<td>Methylation studies**</td>
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<td></td>
<td>Plant tissue*</td>
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<td>Mutation detection</td>
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<tr>
<td></td>
<td>Plasmid</td>
<td></td>
<td>Silencing RNA (siRNA)</td>
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<tr>
<td></td>
<td>Purified gDNA and cDNA</td>
<td></td>
<td>Singleplex and duplex reactions</td>
</tr>
<tr>
<td></td>
<td>Soil*</td>
<td></td>
<td>Viral/pathogen low-copy detection</td>
</tr>
<tr>
<td><strong>SsoAdvanced™ Universal Probes Supermix</strong></td>
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<tr>
<td><strong>SsoAdvanced™ Universal Inhibitor-Tolerant SYBR® Green Supermix</strong></td>
<td>Blood spot*</td>
<td>AT- and GC-rich regions</td>
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<tr>
<td></td>
<td>Cell lysate*</td>
<td>50–250 bp amplicons</td>
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<td>Crude extraction*</td>
<td>Increased secondary structure</td>
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<td></td>
<td>Plant tissue*</td>
<td>Longer amplicons, &gt;250 bp</td>
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<td></td>
<td>Formalin-fixed, paraffin-embedded (FFPE)</td>
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<td>Laser capture microdissection (LCM)</td>
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<td></td>
<td>Plant extract</td>
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<td></td>
<td>Plant tissue*</td>
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<td></td>
<td>Plasmid</td>
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<td></td>
<td>Purified gDNA and cDNA</td>
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<td>Soil*</td>
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<tr>
<td><strong>iTaq™ Universal SYBR® Green Supermix</strong></td>
<td>Plasmid</td>
<td>40–60% GC-rich regions</td>
<td>Gene expression analysis</td>
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<tr>
<td></td>
<td>Purified gDNA and cDNA</td>
<td>50–250 bp amplicons</td>
<td>Genotyping</td>
</tr>
<tr>
<td><strong>iTaq™ Universal Probes Supermix</strong></td>
<td></td>
<td></td>
<td>IncRNA and miRNA expression analysis</td>
</tr>
<tr>
<td><strong>iTaq™ Universal SYBR® Green One-Step Kit</strong></td>
<td>Cell lysates</td>
<td>AT- and GC-rich regions</td>
<td>Mutation detection</td>
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<tr>
<td></td>
<td>Purified RNA</td>
<td>50–250 bp amplicons</td>
<td>Pathogen detection</td>
</tr>
<tr>
<td><strong>iTaq™ Universal Probes One-Step Kit</strong></td>
<td></td>
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<td>Singleplex and duplex reactions</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>siRNA knockdown</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Viral/pathogen low-copy detection</td>
</tr>
</tbody>
</table>

* SsoAdvanced™ Universal Inhibitor-Tolerant SYBR® Green Supermix can be used with blood spots, cell lysates, crude extractions, plant tissues, and soil samples.

** Non-bisulfited DNA only.

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View the Universal Real-Time PCR Reagents App for more information.
SsoAdvanced™ Universal Supermixes

Sso7d Fusion Enzyme Technology

SsoAdvanced Universal Supermixes employ our patented* Sso7d fusion enzyme technology for higher processivity, increased PCR inhibitor tolerance, and robust performance with challenging templates and target sequences in real-time qPCR.

SsoAdvanced Universal Supermixes allow you to:

- **Use any real-time PCR system** — the universal reference dye in these supermixes enables ROX normalization of qPCR data regardless of the ROX requirements of the qPCR system.
- **Employ PrimePCR™ Assays and Panels** — validated for use with SsoAdvanced Universal Supermixes.
- **Achieve superior real-time PCR results under various conditions** — our robust formulation delivers consistent performance in fast and standard cycling across a broad range of reaction conditions, primer concentrations, and temperature ranges.
- **Carry out high-performance singleplex and duplex reactions** — the Sso7d fusion polymerase and advanced formulation enable robust performance in singleplex or duplex real-time PCR reactions, providing the highest data precision and allowing cost and time savings when combining 2 assays in a single well.
- **Increase qPCR sensitivity and efficiency of detection from compromised samples** — the Sso7d fusion polymerase has increased resistance to a wide variety of PCR inhibitors, which helps provide ultimate sensitivity and performance.

SsoAdvanced Universal Supermixes include:

- SsoAdvanced™ Universal SYBR® Green Supermix
- SsoAdvanced Universal Probes Supermix
- SsoAdvanced™ Universal Inhibitor-Tolerant SYBR® Green Supermix

The dsDNA binding protein, Sso7d, stabilizes the polymerase-template complex, increases processivity, and provides greater speed and reduced reaction times compared to conventional DNA polymerases. Sso7d fusion polymerases are significantly more resistant to PCR inhibitors, making the SsoAdvanced Supermixes ideal choices for challenging applications, such as direct qPCR, without the need for sample preparation.

* U.S. patents 6,627,424; 7,541,170; and 7,560,260.
SsoAdvanced™ Universal Supermixes

SsoAdvanced™ Universal SYBR® Green and Probes Supermixes
SsoAdvanced Universal Supermixes employ our patented* Sso7d fusion enzyme technology for higher processivity, increased PCR inhibitor tolerance, and robust performance with challenging templates and target sequences in real-time PCR.

SsoAdvanced™ Universal SYBR® Green Supermix

A. Optimal PCR Sensitivity

Optimal PCR sensitivity and specificity with SsoAdvanced™ Universal SYBR® Green Supermix. A, a PrimePCR™ Assay targeting β2 microglobulin, using 250, 125, 62.5, 31.25, 15.625, and 7.8125 cells/20 µl reaction was performed on a CFX96™ Real-Time PCR Detection System and yielded optimal PCR sensitivity; B, melt curve analysis displays enhanced PCR. RFU, relative fluorescence units.

B. Superior PCR Specificity

Superior qPCR performance in a duplex reaction (A) and single-copy detection (B) with SsoAdvanced Universal Probes Supermix. A, predesigned TaqMan Gene Expression Assays in a duplex reaction with 10 ng–100 fg cDNA, using an 18S rRNA assay (■), and 100 ng–10 pg cDNA, using a TFRC assay (●), were performed on an Applied Biosystems ViiA 7 System; B, a gDNA assay targeting IL-1β, with gDNA serially diluted to a calculated single copy (▲), was performed on a CFX96 Real-Time PCR Detection System. RFU, relative fluorescence units; ΔRn, baseline-corrected normalized reporter.

* U.S. patents 6,627,424; 7,541,170; and 7,560,260.
SsoAdvanced™ Universal Supermixes

SsoAdvanced™ Universal Inhibitor-Tolerant SYBR® Green Supermix

SsoAdvanced™ Universal Inhibitor-Tolerant SYBR® Green Supermix is a high-performance real-time PCR supermix specifically formulated for use with difficult target sequences in a wide range of challenging samples, including crude lysates. The dsDNA binding protein, Sso7d, stabilizes the polymerase-template complex, providing superior PCR inhibitor tolerance, increased processivity and specificity, and greater speed without affecting PCR sensitivity, efficiency, or reproducibility.

- Skip RNA and DNA extraction by using crude samples — lysates from plants, seeds, cells, bacteria, or FFPE samples
- Power through PCR inhibitors
- Obtain high-quality data with fast cycling across a broad range of reaction conditions, primer concentrations, and temperatures
- Decrease time to results without compromising qPCR data quality — rapid polymerization kinetics and instant polymerase activation
- Obtain better results with Bio-Rad’s PrimePCR™ Assays — real-time PCR assays are expertly designed and wet-lab validated for the human, mouse, and rat genomes to ensure optimal assay performance
- Increase cDNA loading — add up to 20% cDNA from our iScript™ Advanced cDNA Synthesis Kit for RT-qPCR or iScript Reverse Transcription Supermix for RT-qPCR
- Use with any real-time PCR system

Applications and Uses
- Environmental monitoring
- Gene expression
- Genetically modified organism (GMO) testing
- Mutation detection
- Pathway analysis
- Single nucleotide polymorphism (SNP) genotyping
- Viral and bacterial detection

PCR inhibitor tolerance testing with SsoAdvanced™ Universal Inhibitor-Tolerant SYBR® Green Supermix against Quanta’s PerfeCTa® SYBR® Green FastMix and Clontech’s Terra™ qPCR Direct SYBR® Premix. Each qPCR reaction was performed using a challenging long (226 bp) human gDNA assay targeting GAPDH and 5 ng of human gDNA. The values represent the maximum percentage and concentration for which a ≤1 quantification cycle (Cq) delay was noted. Any delay >1 Cq was deemed unacceptable for inhibitor tolerance. —, not tested.
**SsoAdvanced™ PreAmp Supermix**

PreAmp provides the ultimate in unbiased, target-specific preamplification from limited nucleic acids, using up to 400 qPCR or end-point assays and enabling more downstream reactions for screening of respective targets.

**Features of SsoAdvanced PreAmp Supermix**
- Unrivaled, with half the bias specification of the competition
- Requires as little as 100 pg of gDNA or cDNA
- Validated for SYBR® Green and probe chemistries
- Optimized for PrimePCR™ PreAmp Assays
- Improved performance using TaqMan and custom assays
- Superior sensitivity and efficiency
- Economically priced

**Compatible Sample Types**
- FFPE samples
- LCM samples
- Plant samples
- Rare samples
- Single cells
- Small biopsies
- Sorted cells
- Stem cells

**Comparison of PreAmp Products**

**A. Bio-Rad**

Twenty-four qPCR probe assays with their respective qPCR reagents were used to evaluate the level of bias.

**B. Life Technologies**

PrimePCR PreAmp Probe Assays, SsoAdvanced PreAmp Supermix, and SsoAdvanced Universal Probes Supermix

**C. Bio-Rad**

Ninety qPCR SYBR® Green assays with their respective qPCR reagents were used to evaluate the level of bias.

**D. Quanta BioSciences**

Perfectq PreAmp 5x SuperMix and Perfectq SYBR® Green FastMix

**Bias Quantification Score**

A bias quantification (BQ) score provides a numerical value for the level of bias observed in a preamplification reaction. It is based on comparing the theoretical difference between a sample with and without PreAmp compared to the observed (actual) difference between a sample with and without PreAmp. Zero indicates no bias, and the farther from zero the BQ score becomes, the greater the bias introduced.
SsoAdvanced™ PreAmp Supermix

This guide provides a comparison of PreAmp products.

<table>
<thead>
<tr>
<th>Property</th>
<th>SsoAdvanced PreAmp Supermix</th>
<th>TaqMan PreAmp Master Mix (Life Technologies)</th>
<th>PerfeCTa PreAmp 5x SuperMix (Quanta BioSciences)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of preamplification targets</td>
<td>400</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>BQ score</td>
<td>90% ≤ 0.75*</td>
<td>90% ≤ 1.50</td>
<td>90% ≤ 1.50</td>
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<tr>
<td>Sensitive detection of low-level target genes</td>
<td>***</td>
<td>**</td>
<td>•</td>
</tr>
<tr>
<td>High efficiency, even for difficult amplicons</td>
<td>***</td>
<td>**</td>
<td>**</td>
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<tr>
<td>Validated assays</td>
<td>PrimePCR™ PreAmp, TaqMan</td>
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<td>Unknown</td>
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<td>Chemistry</td>
<td>SYBR® Green and probes</td>
<td>Probes</td>
<td>SYBR® Green and probes</td>
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<td>Number of reactions</td>
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<td>40 x 50 µl</td>
<td>40 x 50 µl</td>
</tr>
</tbody>
</table>

• Fair
** Good
*** Excellent

SsoAdvanced PreAmp Supermix provides optimal preamplification of low input cDNA.

Preamplification of a panel of 22 neuronal stem cell targets treated with retinoic acid to alter the stemness of NTERA2 cell lines with input cDNA of 10 ng, 1 ng, and 100 pg per preamplification reaction across six time points (days). A 1 µg no-preamplification control was used in the experiment. Results from NANOG (A, a high expressor), PAX6 (B, a medium expressor), and NEUROG2 (C, a low expressor) show consistent expression levels regardless of the input cDNA.

For more information, download or request bulletin 6576.
iTaq™ Universal Supermixes

iTaq Universal Supermixes are ready-to-use reaction master mixes for SYBR® Green– or probe-based assays. These supermixes contain an advanced buffer formulation for optimal qPCR performance.

- Quickly start your experiment within 30 sec with antibody-mediated hot-start iTaq DNA Polymerase for rapid activation
- Obtain reliable and reproducible gene expression analysis results
- Achieve consistent results across real-time instrument platforms under fast or standard conditions
- Utilize any real-time PCR system, with its blend of passive reference dyes
- Employ PrimePCR™ Assays and Panels — validated for use with iTaq Universal Supermixes

Use iTaq Universal Supermixes for:
- Plasmid, purified gDNA, and cDNA samples
- Singleplex or duplex assays for a wide variety of targets (duplex for probed-based assays)
- Gene expression analysis, genotyping, mutation detection, pathogen detection

iTaq™ Universal SYBR® Green Supermix

- Contains an advanced buffer formulation with SYBR® Green I for optimal qPCR performance
- Provides a way to amplify gDNA and difficult amplicons with superior efficiency
- Applications and uses include gene expression analysis, genotyping, detection, and pathogen detection

Matchless Precision and Accuracy

Superior accuracy and precision with iTaq™ Universal SYBR® Green Supermix ensures production of high-quality data. An ACTB cDNA assay was performed on a CFX96 ™ Real-Time PCR Detection System using iTaq™ Universal SYBR® Green Supermix, which produced 1.33-fold discrimination. RFU, relative fluorescence units.
iTaq™ Universal Supermixes

iTaq Universal Probes Supermix

- Carry out high-performance singleplex and duplex reactions — robust performance in singleplex or duplex real-time PCR reactions enables increased data precision and saves samples, reagents, and time
- Achieve superior results under various conditions — broad range of cycling conditions, primer concentrations, temperature ranges, sample types, and target sequences can be applied
- Decrease run times and time to results without compromising qPCR data quality — provides rapid polymerization kinetics and instant polymerase activation for run times less than 40 minutes

Dynamic range of iTaq Universal Probes Supermix. An ACTB cDNA assay was performed on an Applied Biosystems 7900 System and produced standard curve $R^2 = 0.999$ and efficiency $= 99.4\%$ with a 6-log dynamic range. ΔRn, baseline-corrected normalized reporter.

Comparison of dynamic range using iTaq Universal Probes Supermix vs. TaqMan Fast Universal PCR Master Mix (Life Technologies). An ACTB cDNA assay was performed on an Applied Biosystems 7900 System. iTaq Universal Probes Supermix demonstrates a 6-log dynamic range with earlier Cq values and greater sensitivity compared to TaqMan Fast Universal PCR Master Mix, which demonstrates a 5-log dynamic range and delayed Cq values. ΔRn, baseline-corrected normalized reporter.
 iTaq™ Universal One-Step Kits

iTaq Universal One-Step Kits are a fast and convenient solution for real-time PCR, using the powerful combination of iScript™ RNase H+ MMLV Reverse Transcriptase and hot-start iTaq DNA Polymerase in one reaction. Select from SYBR® Green– or probe-based assays for flexibility.

- Achieve superior gene expression results under various cycling conditions — our robust formulation delivers consistent performance in standard or fast cycling conditions, primer concentrations, sample and target types, and temperature ranges, allowing cost and time savings
- Improve PCR efficiency and obtain wider dynamic range, superior sensitivity and specificity, and inhibitor tolerance — even with cell lysates — without affecting performance
- Use any ROX dependent or independent real-time PCR system — the universal reference dye in these supermixes enables ROX normalization of qPCR data regardless of the ROX requirements of the qPCR system
- Minimize handling and contamination risk — perform cDNA synthesis and qPCR in 1 tube

iTaq™ Universal SYBR® Green One-Step Kit

- Benefit from enhanced efficiency, specificity, and sensitivity — our patented inhibitor reducer prevents the interference of the RT enzyme with the DNA polymerase during the replication of cDNA
- Obtain better results with qPCR assays — simplify your workflow and experimental design using our wet-lab validated PrimePCR™ Assays; each assay for the human, mouse, and rat genomes was experimentally tested for optimal efficiency, specificity, sensitivity, and linear dynamic range

Superior Dynamic Range and Sensitivity

A. Bio-Rad

B. Life Technologies

Superior dynamic range and sensitivity with iTaq™ Universal SYBR® Green One-Step Kit. A 120 bp human FAS assay with an input of 500 ng–50 pg total RNA was performed on a ViiA 7 Real-Time PCR System (Life Technologies). A, iTaq™ Universal SYBR® Green One-Step Kit; B, Power SYBR® Green RNA-to-Ct, 1-Step Kit (Life Technologies). Note the delayed threshold cycle (Ct) values, loss of dynamic range, and compression of input 500 ng total RNA using the Power SYBR® Green RNA-to-Ct, 1-Step Kit. This demonstrates RT-qPCR inhibition with higher input RNA and lack of low-level target expression detection. ∆Rn, baseline-corrected normalized reporter.

* U. S. patent 8,338,094.
iTač™ Universal One-Step Kits

iTač Universal Probes One-Step Kit

- **Obtain superior results with singleplex and multiplex reactions** — advanced formulation enables the simultaneous amplification of up to 3 targets, resulting in higher data precision with fewer pipetting steps and reduced sample usage

- **High-throughput real-time PCR screening and validation** — simplified workflow and reduced cycling times enable screening and validation of the greatest number of samples and targets in the shortest period of time

Optimal RT-qPCR Efficiency and Sensitivity with an AT-Rich and Long Amplicon

**A. Bio-Rad**

61% AT-rich, 248 bp HPRT assay with 100 ng–10 pg RNA

\[ R^2 = 0.999 \]

Efficiency = 94.1%

**B. Life Technologies**

61% AT-rich, 248 bp HPRT assay with 100 ng–10 pg RNA

\[ R^2 = 0.996 \]

Efficiency = 93.3%

Optimal RT-qPCR efficiency and sensitivity with iTaq Universal Probes One-Step Kit. A 61% AT-rich, long, 248 bp HPRT assay with an input of 100 ng–10 pg RNA was performed on a CFX96™ Real-Time PCR Detection System. A, iTaq Universal Probes One-Step Kit; B, TaqMan RNA-to-C\(_\text{T}\) 1-Step Kit (Life Technologies) produced delayed Cq values with less than optimal data precision for the technical replicates. RFU, relative fluorescence units.

Superior RT-qPCR Performance in a Triplex Reaction

**A. Bio-Rad**

Dye | Logs | Efficiency, % | Standard Curve, \( R^2 \)
--- | --- | --- | ---
FAM | 5 | 106 | 0.998
HEX | 4 | 101 | 0.999
Quasar | 6 | 103 | 0.998

**B. Life Technologies**

Dye | Logs | Efficiency, % | Standard Curve, \( R^2 \)
--- | --- | --- | ---
FAM | 4 | 96.7 | 0.999
HEX | 4 | 96.7 | 0.999
Quasar | 4 | 95.6 | 0.999

Superior RT-qPCR performance in a triplex reaction with iTaq Universal Probes One-Step Kit. An XPO (FAM), TUB (HEX), and GAPDH (Quasar) assay set with an input of 100 ng–10 fg RNA was performed on a CFX96 Real-Time PCR Detection System. A, iTaq Universal Probes One-Step Kit exhibited the greatest dynamic range across all three assays, which indicates a high degree of sensitivity. In addition, the iTaq Kit demonstrated the lowest standard deviations for technical replicates. B, TaqMan RNA-to-C\(_\text{T}\) 1-Step Kit from Life Technologies did not perform as well, exhibiting 4 logs of dynamic range for each assay (lower sensitivity) and higher standard deviations for technical replicates (lower data precision). RFU, relative fluorescence units.
**iQ™ Supermixes**

iQ Supermixes contain hot-start iTaq™ DNA Polymerase, deoxynucleoside triphosphates (dNTPs) qualified for qPCR, and buffer at concentrations optimal for real-time PCR assays. These supermixes can be used across a wide dynamic range of human genomic and plasmid DNA concentrations.

**iQ Multiplex Powermix**
- Robust supermix formulated for sensitive and efficient multiplex qPCR
- Reliable quantification of up to 4 targets (expression levels can vary up to $10^6$-fold between target genes) or up to 5 targets
- Linearity over 6 orders of magnitude of input cDNA and 4 orders of magnitude of input genomic DNA
- Suitable for a wide variety of applications, including gene expression analysis, SNP genotyping, SNP analysis, GMO detection, and viral load detection

**iQ™ SYBR® Green Supermix**
- Analysis of low-, medium-, and high-abundance target genes with superior sensitivity and efficiency
- Formulated for maximum SYBR® Green I stability and performance in a wide variety of real-time PCR instruments

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**Accurate and Precise qPCR Results**

<table>
<thead>
<tr>
<th>Dye</th>
<th>Efficiency, %</th>
<th>Standard Curve, $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAM</td>
<td>97.8</td>
<td>1.000</td>
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<tr>
<td>HEX</td>
<td>98.9</td>
<td>1.000</td>
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<tr>
<td>Texas Red</td>
<td>99.7</td>
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<tr>
<td>Cy5</td>
<td>98.1</td>
<td>1.000</td>
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<tr>
<td>Quasar</td>
<td>99.8</td>
<td>1.000</td>
</tr>
</tbody>
</table>

iQ Multiplex Powermix produces highly accurate and precise 5-plex qPCR results in a single well reaction across 6 logs of dynamic range. RFU, relative fluorescence units.

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For more information, download or request bulletins 2764 and 5348.
**Precision Melt Supermix**

Precision Melt Supermix is a superior high resolution melt (HRM) supermix that delivers optimal discrimination for numerous applications.

- Sensitive and specific discrimination of class I–IV SNPs across a broad range of amplicons
- Ideal solution for insertions or deletions >6 base pairs
- Accurate detection of the percentage of CpG methylation status for epigenetic studies
- Ideal for mutation screening of small mutations or using a primer walking approach for larger regions
- Cost-saving application for upstream and downstream next-generation sequencing (NGS) prescreens and postvalidations
- Exceptional room temperature stability for high-throughput HRM studies
- Optimized formulation containing EvaGreen dye delivers robust PCR and HRM performance

**Applications and Uses**

- Genotyping
- Methylation studies
- Mutation detection
- NGS screening
- Species identification

**Exceptional Stability**

Specific amplification and accurate discrimination of a class IV SNP (84 bp amplicon) from mouse gDNA was performed on a CFX384™ Real-Time PCR Detection System either 0 hr (A) or 48 hr (B) after reaction setup. Wild type (■), heterozygote (■), and homozygous mutant (■) are shown in the difference plots normalized to wild-type samples. Total run time including melt curve = 150 min. RFU, relative fluorescence units.

**Accurate Methylation Detection**

Mixtures of methylated and unmethylated human gDNA of varying ratios were analyzed using HRM on a CFX384 Real-Time PCR Detection System. Increasing amounts of methylated DNA were analyzed for methylation of the human RARB2 gene. The genomic region contains seven CpG sites and is 88 bp in length. Total run time including melt curve = 190 min. RFU, relative fluorescence units.

For more information, download or request bulletin 6137.
Precision Blue™ Real-Time PCR Dye

Precision Blue Real-Time PCR Dye is a concentrated, ready-to-use reagent that enhances the visibility of real-time PCR reactions. This robust, versatile reagent is formulated for a wide range of real-time PCR applications.

- Facilitates precise pipetting and accurate reaction tracking when loading tubes or plates
- Helps increase qPCR accuracy and reproducibility
- Decreases setup time without compromising qPCR data quality
- Useful for loading white or 384-well plates

Precision Blue Dye performs well and is compatible with:
- Bio-Rad’s wet-lab validated PrimePCR™ Assays for the human, mouse, and rat genomes
- Bio-Rad’s universal real-time supermixes and one-step kits
- Fluorogenic probes and SYBR® Green chemistries

Pipetting a reaction mix into a PrimePCR Assay Plate.
iProof™ High-Fidelity DNA Polymerase
iProof High-Fidelity DNA Polymerase is composed of a unique Pyrococcus-like proofreading enzyme fused to a dsDNA binding protein, Sso7d. This results in a thermostable polymerase that accurately amplifies long products from a variety of DNA templates.

In comparison to reactions using Taq polymerase, iProof DNA Polymerase features:

- **High fidelity** — iProof DNA Polymerase is 52-fold more accurate
- **Speed** — high processivity dramatically reduces extension time (15–30 sec/kb) and overall reaction times
- **Successful amplification of long products with higher yields** — fragments up to 37 kb are amplified in less time and with less enzyme (0.25–1 U/reaction)

iProof DNA Polymerase is available in three convenient formats:

- Stand-alone enzyme
- Easy-to-use master mix
- PCR kit complete with controls

iTaq™ DNA Polymerase
- Antibody-mediated hot-start DNA polymerase for quick 3 min activation at 95°C
- Polymerase prevents nonspecific amplification and primer-dimers in both PCR and real-time PCR applications

For more information, download or request bulletins 2779 and 5211.
Standard PCR Reagents

Amplification of Long Templates

A

BAC DNA (37 kb)

B

Human genomic DNA (28 kb)

iProof™ High-Fidelity DNA Polymerase amplifies long templates with high yields. A, various fragments up to 37 kb in length were amplified from BAC DNA using a combined annealing/extension step of 10 min per cycle and 30 U/ml of iProof Polymerase. B, various sequences up to 28.8 kb were amplified directly from human genomic DNA using 30 U/ml of iProof Polymerase in GC buffer with a combined annealing/extension time of 10 min per cycle. BAC, bacterial artificial chromosome.

Shorter Overall Reaction Times

iProof High-Fidelity DNA Polymerase demonstrates unrivaled speed, leading to dramatically shorter overall reaction times. The reaction protocol for iProof Polymerase was compared to the recommended protocols for two competing polymerases. Each protocol was designed to amplify 1, 8, and 15 kb products in 30 cycles. Reactions with iProof Polymerase used a two-step protocol with a combined annealing and extension step, while the other reactions used three-step protocols with the minimum recommended extension times. Overall reaction times include temperature ramping times.
## Reagent Compatibility with Instruments

<table>
<thead>
<tr>
<th>Real-Time PCR Instrument</th>
<th>SYBR® Green Supermixes</th>
<th>Probes Supermixes</th>
<th>One-Step Kits for RT-qPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-Rad</td>
<td>✔ ✔ ✔ ✔ ✔ ✔ ✔ ✔ ✔ ✔</td>
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<td>CFX96™, CFX96 Touch™, CFX96 Touch Deep Well, CFX384™, CFX384 Touch™, CFX Connect™</td>
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<td>iQ™, iQ™5, MyiQ™, MyQ™2</td>
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<td>MiniOpticon™, DNA Engine Opticon® I and II</td>
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<td>LightScanner 32</td>
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- ✔ Recommended for use as is
- ◆ ROX reference setting must be turned off
- ▲ BSA must be added according to instrument specifications
- — Not compatible
Ordering Information

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Description</th>
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<td>SsoAdvanced Universal SYBR Green Supermix, 2 ml (2 x 1 ml vials), 200 x 20 μl reactions</td>
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<td>172-5271</td>
<td>SsoAdvanced Universal SYBR Green Supermix, 5 ml (5 x 1 ml vials), 500 x 20 μl reactions</td>
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<td>iTaq Universal SYBR Green Supermix, 50 ml (10 x 5 ml vials), 5,000 x 20 μl reactions</td>
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<tr>
<td>172-5130</td>
<td>iTaq Universal Probes Supermix, 2 ml (2 x 1 ml vial), 200 x 20 μl reactions</td>
</tr>
<tr>
<td>172-5131</td>
<td>iTaq Universal Probes Supermix, 5 ml (5 x 1 ml vial), 500 x 20 μl reactions</td>
</tr>
<tr>
<td>172-5132</td>
<td>iTaq Universal Probes Supermix, 10 ml (10 x 1 ml vials), 1,000 x 20 μl reactions</td>
</tr>
<tr>
<td>172-5134</td>
<td>iTaq Universal Probes Supermix, 25 ml (5 x 5 ml vials), 2,500 x 20 μl reactions</td>
</tr>
<tr>
<td>172-5135</td>
<td>iTaq Universal Probes Supermix, 50 ml (10 x 5 ml vials), 5,000 x 20 μl reactions</td>
</tr>
<tr>
<td>172-5120</td>
<td>iTaq Universal One-Step Kits</td>
</tr>
<tr>
<td>172-5121</td>
<td>iTaq Universal SYBR Green One-Step Kit, 1 ml (1 x 1 ml vial), 100 x 20 μl reactions</td>
</tr>
<tr>
<td>172-5122</td>
<td>iTaq Universal SYBR Green One-Step Kit, 5 ml (5 x 1 ml vials), 500 x 20 μl reactions</td>
</tr>
<tr>
<td>172-5124</td>
<td>iTaq Universal Probes One-Step Kit, 1 ml (1 x 1 ml vial), 100 x 20 μl reactions</td>
</tr>
<tr>
<td>172-5125</td>
<td>iTaq Universal Probes One-Step Kit, 5 ml (5 x 1 ml vial), 500 x 20 μl reactions</td>
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Catalog # Description

<table>
<thead>
<tr>
<th>IQ Supermixes</th>
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<tbody>
<tr>
<td>172-5848</td>
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<td>172-5849</td>
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<td>170-8880</td>
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<td>170-8882</td>
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<td>170-8880</td>
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<td>170-8882</td>
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Standard PCR Reagents

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Description</th>
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<tbody>
<tr>
<td>172-5300</td>
<td>iProof High-Fidelity DNA Polymerase, 20 U, 2 U/μl</td>
</tr>
<tr>
<td>172-5301</td>
<td>iProof High-Fidelity DNA Polymerase, 100 U, 2 U/μl</td>
</tr>
<tr>
<td>172-5302</td>
<td>iProof High-Fidelity DNA Polymerase, 500 U, 2 U/μl</td>
</tr>
<tr>
<td>172-5310</td>
<td>iProof HF Master Mix, 0.04 U/μl, 100 x 50 μl reactions</td>
</tr>
<tr>
<td>172-5320</td>
<td>iProof GC Master Mix, 0.04 U/μl, 100 x 50 μl reactions</td>
</tr>
<tr>
<td>172-8858</td>
<td>ROX Passive Reference Dye, 0.5 ml</td>
</tr>
<tr>
<td>170-8870</td>
<td>iTaq DNA Polymerase, 250 U, 5 U/μl</td>
</tr>
<tr>
<td>170-8875</td>
<td>iTaq DNA Polymerase, 5,000 U, 5 U/μl</td>
</tr>
<tr>
<td>170-8874</td>
<td>dNTP Mix, 100 U</td>
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Additional Real-Time PCR Reagents

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Description</th>
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<tbody>
<tr>
<td>172-5110</td>
<td>Precision Melt Supermix, 2 ml (2 x 1 ml vials), 200 x 20 μl reactions</td>
</tr>
<tr>
<td>172-5112</td>
<td>Precision Melt Supermix, 10 ml (10 x 1 ml vials), 1,000 x 20 μl reactions</td>
</tr>
<tr>
<td>172-5555</td>
<td>Precision Blue Real-Time PCR Dye, 55 μl (1 x 1 ml vial), 500 x 20 μl reactions</td>
</tr>
</tbody>
</table>

Visit www.bio-rad.com/web/RT-PCRreagents for additional real-time PCR reagents.
A large selection of PCR tubes, PCR plates, seals, and accessories are precisely manufactured for optimal fit and cycling performance in thermal cyclers and real-time PCR systems.

- Produced in Class 10,000 or 100,000 cleanroom environment
- Certified to be free of DNase, RNase, and human gDNA
- Extremely uniform wells reduce well-to-well variability in real-time PCR
- Warp-free Hard-Shell® Plates are designed for optimum performance with automation
## Instrument Compatibility

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Individual High-Profile</th>
<th>Strips High-Profile</th>
<th>Strips Low-Profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalog #</td>
<td>TBI-0201, TFI-0201, TWI-0201</td>
<td>TBS-xxxx, TBC-xxxx</td>
<td>TLS-xxxx</td>
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</tbody>
</table>

### Thermal Cycler

- **Bio-Rad® C1000™**, C1000 Touch™, S1000™
- **Bio-Rad® DNA Engine™**, DNA Engine Tetrad®, DNA Engine Tetrad 2, DNA Engine Dyad®, Dyad Disciple™, PTC-100®
- **Bio-Rad® T100™**, MyCycler™
- **Bio-Rad® iCycler®**
- **Bio-Rad® MJ Mini™**
- **Applied Biosystems 0.2 ml Tube Cyclers (2720, 9700, Veriti)**
- **Applied Biosystems 0.1 ml Tube Cyclers (9800 Fast, Veriti Fast)**
- **Eppendorf Mastercycler Series**

### Real-Time PCR Instrument

- **Bio-Rad® CFX96™, CFX96 Touch™, CFX96 Touch Deep Well, CFX Connect™**
- **Bio-Rad® iCycler IQ™, IQ™5, MyIQ™, MyIQ™2**
- **Bio-Rad® Chromo4™**
- **Bio-Rad® DNA Engine Opticon®, Opticon 2**
- **Bio-Rad® MiniOpticon™**
- **Applied Biosystems Standard Systems (7300, 7500, 7900HT)**
- **Applied Biosystems Fast Systems (7500 Fast, 7900HT Fast, StepOne, StepOnePlus)**
- **Eppendorf Mastercycler ep realplex**
- **Stratagene (Agilent) Mx Series**
- **QIAGEN/Corbett Rotor-Gene**

### Recommended

- ✔️

### Compatible

- ▲

* The MiniOpticon Real-Time PCR Detection System is factory calibrated for white tubes and white-well plates. White plastics are recommended due to their superior signal-to-noise ratio. Using clear tubes or clear-well plates on this instrument will require user calibration.
# 5 PCR PLASTIC CONSUMABLES

## Instrument Compatibility

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>96- and 48-Well Plates</th>
<th>384-Well Plates</th>
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<tbody>
<tr>
<td></td>
<td>Hard-Shell™ Semi-Skirted High-Profile</td>
<td>Hard-Shell™ Skirted Low-Profile</td>
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<tr>
<td>HSS-9xxx</td>
<td>✔</td>
<td>▲</td>
</tr>
<tr>
<td>HSP-9xxx</td>
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<tr>
<td>HSL-9xxx</td>
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<td>HSR-99xx</td>
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<tr>
<td>MLP-xxxx</td>
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<tr>
<td>MLL-xxxx</td>
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<td>223-9441</td>
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</table>

### Thermal Cyclers

- **Bio-Rad® C1000™, C1000 Touch™,** S1000™
  - Recommended

- **Bio-Rad® T100™**
  - Compatible

- **Bio-Rad® DNA Engine®, DNA Engine Tetrad®, DNA Engine Tetr 2, DNA Engine Dyad®, Dyad Disciple®, PTC-100®**
  - Recommended

- **Bio-Rad® iCycler®, MyCycler™**
  - Except MyCycler

- **Bio-Rad® MJ Mini™**
  - Compatible

- **Applied Biosystems 0.2 ml Tube Cyclers (2720, 9700, Veriti)**
  - Compatible

- **Applied Biosystems 0.1 ml Tube Cyclers (9800 Fast, Veriti Fast)**
  - Compatible

- **Applied Biosystems 384-Well Cyclers (9700, Veriti)**
  - Compatible

- **Eppendorf Mastercycler Series**
  - Recommended

### Real-Time PCR Instrument

- **Bio-Rad® CFX96®, CFX384 Touch™,** CFX Connect™, CFX384™, CFX384 Touch™
  - Recommended

- **Bio-Rad® CFX96 Touch Deep Well**
  - Compatible

- **Bio-Rad® iCycler IQ™, IQ® 5, MyIQ®, MyIQ® 2**
  - Compatible

- **Bio-Rad® Chromo4™**
  - Compatible

- **Bio-Rad® DNA Engine Opticon®, Opticon 2, MiniOpticon™**
  - Recommended

- **Applied Biosystems Standard Systems (7300, 7500, 7900HT, QuantStudio 12K Flex, ViA 7)**
  - Compatible

  - Compatible

- **Eppendorf Mastercycler ep realplex**
  - Compatible

- **Stratagene (Agilent) Mx Series**
  - Compatible

- **Roche LightCycler 96, 480**
  - Compatible

### Other Instruments

- **Applied Biosystems DNA Sequencers (3100, 3700, 3730)**
  - Compatible

- **BioFire LightScanner**
  - Compatible

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* CFX384, CFX384 Touch, and MiniOpticon Real-Time PCR Detection Systems are factory calibrated for white tubes and white-well plates. White plastics are recommended due to their superior signal-to-noise ratio. Using clear tubes or clear-well plates on these instruments will require user calibration.
PCR Tubes and Strips

0.2 and 0.5 ml Individual PCR Tubes
- High-profile PCR tubes with double-locking caps for a stronger seal during cycling
- Flat, frosted caps are easy to label
- Options with attached caps for greater convenience and lower risk of contamination

High-Profile PCR Tube Strips
- Thin-walled tubes for superior heat transfer
- 8- and 12-tube strips for use on 48- or 96-well sample blocks
- Available in a variety of colors for easy sample tracking
- Maximum volume: 300 µl

Low-Profile PCR Tube Strips
- Lower height reduces the potential for condensation
- Designed to allow greater light capture in fluorescence assays
- Opaque white color option maximizes detection signal
- Maximum volume: 200 µl

Flat and Domed Cap Strips for PCR Tubes and PCR Plates
- Ultraclear flat cap strips are ideal for qPCR
- Designed for extremely tight sealing for thermal cycling and storage (−20 and 4°C)
- Flat caps have optimal light transmittance for real-time PCR on PCR tubes or plates
- Great option to save plates if fewer wells are needed during PCR
PCR Plates

Multiplate™ 96-Well and 48-Well Unskirted PCR Plates
- Single-component polypropylene allows low protein binding and excellent sample retention
- High-profile (20.7 mm) and low-profile (15.5 mm) options allow broad real-time PCR instrument compatibility
- White-well option for maximizing real-time PCR detection sensitivity
- Designed to allow easy cutting with scissors (when less than a full plate is needed)

iQ™ High-Profile 96-Well Semi-Skirted PCR Plates
- Semi-skirt design provides a labeling surface for easy sample tracking
- Composition designed to provide stiffness during plate handling
- High-profile (20.7 mm) plate has perforations every 3 columns for convenience (when less than a full plate is needed)
Hard Shell® PCR Plates

Hard-Shell Technology

The patented two-component design of Hard-Shell Plates is specifically engineered to withstand the stresses of thermal cycling. Benefits include:

- Superior stability and flatness allow precise positioning and robotic handling
- Sturdy plate design is ideal for heat sealing methods
- Warp-resistant feature provides durability during automation, high-speed centrifugation, and storage (even −80°C)
- User-readable barcode options for convenient sample tracking in high-throughput settings
- Black alphanumeric labeling for easy well identification
- Footprint and well spacing designed to match ANSI/SBS standard dimensions
- Composition helps prevent DNA binding
- Polypropylene resin allows superior well-to-well uniformity for reliable and reproducible real-time qPCR results

Enhanced Real-Time PCR Sensitivity

For more information, download or request bulletin 5496.
Hard-Shell® PCR Plates

For more information, download or request bulletin 5496.
PCR Plate Sealing

PX1™ PCR Plate Sealer
The PX1 PCR Plate Sealer is a semiautomated heat sealer for consistent and uniform sealing across an entire microplate. Features include:

- Intuitive touch-screen user interface for extreme convenience and ease of use
- Programmable sealing protocols for quick access
- Small footprint suitable for crowded laboratory benches
- Compatible with a variety of heat sealing films and foils and a wide range of PCR plates

Seals Validated for PX1 PCR Plate Sealer

Optically Clear Heat Seal
- Ideal for real-time PCR
- Excellent optical clarity
- Peelable for easy sample retrieval
- Compatible with PCR

Permanent Clear Heat Seal
- Ideal for water bath cycling
- Nonpeelable, nonpierceable seal
- High solvent resistance

Pierceable Foil Heat Seal
- Fully validated for the QX100™ or QX200™ Droplet Digital™ PCR System workflow
- Easily pierceable with a pipet tip
- User friendly — colored stripe clearly identifies sealing surface
- Compatible with PCR

Peelable Foil Heat Seal
- Ideal for low-temperature sample storage
- Can be easily peeled from microplates stored in a –80°C freezer or in liquid nitrogen
- Compatible with PCR
PCR PLASTIC CONSUMABLES

PCR Plate Sealing

Microseal ‘B’ Adhesive Seals, Optically Clear
- Strongest adhesive-based optically clear sealing option designed for real-time PCR plates
- Withstands multiple storage or transport temperatures (–40 to 110°C)

Microseal ‘C’ Optical Seals
- Optically clear adhesive films designed for tight seals even with wells with raised rims
- Pressure-sensitive adhesive allows easy application during plate sealing
- Designed with superior optical properties for real-time PCR

Microseal ‘F’ Foil
- Aluminum foil allows opaque sealing option for DNA sequencing (ABI 3700 DNA Analyzer) and sample storage
- Acts as a barrier against evaporation in extreme temperatures (–80 to 105°C)
- Pierceable foil for easy sample retrieval

Microseal ‘A’ Film
- A nonoptical, nonadhesive sealing option for quick pressure-based sealing of plates
- Allows easy removal without the risk of aerosol formation, minimizing cross-contamination
- Convenient option for standard PCR needs

Ordering Information

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>181-4000</td>
<td>PX1 PCR Plate Sealer, includes heat sealing instrument, 96-well/384-well plate support block, sealing frame, power cord</td>
</tr>
<tr>
<td>181-4030</td>
<td>Optically Clear Heat Seal, 100 seals</td>
</tr>
<tr>
<td>181-4035</td>
<td>Permanent Clear Heat Seal, 100 seals</td>
</tr>
<tr>
<td>181-4040</td>
<td>Pierceable Foil Heat Seal, 100 seals</td>
</tr>
<tr>
<td>181-4045</td>
<td>Peelable Foil Heat Seal, 100 seals</td>
</tr>
<tr>
<td>MSA-5001</td>
<td>Microseal ‘A’ Film, 50 seals</td>
</tr>
<tr>
<td>MSB-1001</td>
<td>Microseal ‘B’ Adhesive Seals, optically clear, 100 seals</td>
</tr>
<tr>
<td>MSC-1001</td>
<td>Microseal ‘C’ Optical Seals, 100 seals</td>
</tr>
<tr>
<td>MSF-1001</td>
<td>Microseal ‘F’ Foil, 100 seals</td>
</tr>
</tbody>
</table>

Catalog #  Description
MSR-0001  Sealing Roller, for film seals
ADR-3296  Optical Compression Pad, for improved film sealing of 96-well plates in DNA Engine Opticon 2 and Chromo4 Systems
ADR-5001  Pressure Pad, uniformly distributes lid pressure for sealing film
MSO-1001  Optical Film Sealing Kit, for 96-well plates, includes optical compression pad, 100 Microseal ‘B’ Adhesive Seals
223-9444  Optical Sealing Tape, 100 sheets
223-9442  96-Well PCR Plate Sealing Mats, 5
## Ordering Information

### Individual PCR Tubes

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Description</th>
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<tbody>
<tr>
<td>TFI-0201</td>
<td>PCR Tubes with Flat Caps (0.2 ml), clear, 1,000</td>
</tr>
<tr>
<td>TWI-0201</td>
<td>PCR Tubes with Domed Caps (0.2 ml), clear, 1,000</td>
</tr>
<tr>
<td>TBI-0201</td>
<td>PCR Tubes without Caps (0.2 ml), clear, 1,000</td>
</tr>
<tr>
<td>TBI-0501</td>
<td>PCR Tubes with Flat Caps (0.5 ml), clear, 1,000 (2 bags of 500)</td>
</tr>
<tr>
<td>TBI-0502</td>
<td>PCR Tubes with Flat Caps (0.5 ml), clear, 800 (8 bags of 100)</td>
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### PCR Tube Strips

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Description</th>
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<tbody>
<tr>
<td>TBS-0201</td>
<td>8-Tube Strips without Caps, clear, 120 strips (960 PCR tubes)</td>
</tr>
<tr>
<td>TBS-1201</td>
<td>12-Tube Strips without Caps, clear, 100 strips (1,200 PCR tubes)</td>
</tr>
<tr>
<td>TBC-0802</td>
<td>8-Tube Strips and Domed Cap Strips, clear, 20 bags of 12 x 8-tube strips and 12 x 8-cap strips (1,920 PCR tubes and 1,920 caps)</td>
</tr>
<tr>
<td>TBC-1202</td>
<td>12-Tube Strips and Domed Cap Strips, clear, 20 bags of 12 x 12-tube strips and 8 x 12-cap strips (1,920 PCR tubes and 1,920 caps)</td>
</tr>
<tr>
<td>TLS-0801</td>
<td>Low-Profile 8-Tube Strips without Caps, clear, 120 (960 PCR tubes)</td>
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<tr>
<td>TLS-0851</td>
<td>Low-Profile 8-Tube Strips without Caps, white, 120 (960 PCR tubes)</td>
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### Cap Strips

<table>
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<tr>
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<th>Description</th>
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<tbody>
<tr>
<td>TCS-0801</td>
<td>Domed 8-Cap Strips, for PCR tubes and plates, clear, 120</td>
</tr>
<tr>
<td>TCS-1201</td>
<td>Domed 12-Cap Strips, for PCR tubes and plates, clear, 200</td>
</tr>
<tr>
<td>TCS-0803</td>
<td>Optical Flat 8-Cap Strips, for PCR tubes and plates, ultraclear, 120</td>
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</table>

### Capping Tools and Racks

<table>
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<tr>
<th>Catalog #</th>
<th>Description</th>
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<tbody>
<tr>
<td>TRC-9601</td>
<td>PCR Tube Rack, ANSI/SBS standard, white, 10</td>
</tr>
<tr>
<td>TRC-0501</td>
<td>96-Place Racks, with covers, for PCR tubes and unskirted and semi-skirted microplates, assorted colors, 5</td>
</tr>
<tr>
<td>ECT-1000</td>
<td>Easy Cap Tool, ensures tight seal for 0.2 ml PCR tubes or 96-well microplates</td>
</tr>
<tr>
<td>ECT-2000</td>
<td>Strip Cap Tool, for sealing 8- and 12-cap strips on PCR plates or tubes</td>
</tr>
</tbody>
</table>

### PCR Plates

<table>
<thead>
<tr>
<th>Description</th>
<th>Clear Well</th>
<th>White Well</th>
<th>Black Well</th>
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<tbody>
<tr>
<td>Hard-Shell Plates</td>
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</tr>
<tr>
<td>Hard-Shell Low-Profile 96-Well Skirted PCR Plates (50 plates)</td>
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<tr>
<td>White shell</td>
<td>HSP-9601</td>
<td>HSP-9655</td>
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<tr>
<td>Red shell</td>
<td>HSP-9811</td>
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</tr>
<tr>
<td>Yellow shell</td>
<td>HSP-9621</td>
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<tr>
<td>Blue shell</td>
<td>HSP-9631</td>
<td>HSP-9635</td>
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<tr>
<td>Green shell</td>
<td>HSP-9641</td>
<td>HSP-9645</td>
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<tr>
<td>Black shell</td>
<td>HSP-9661</td>
<td>HSP-9665</td>
<td>HSP-9666</td>
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<tr>
<td>White shell, barcoded</td>
<td>HSP-9901</td>
<td>HSP-9955</td>
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<tr>
<td>(Row H and Column 12)</td>
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<tr>
<td>Hard-Shell Low-Profile 96-Well Semi-Skirted PCR Plates (25 plates)</td>
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<tr>
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<td>Black shell</td>
<td>—</td>
<td>HSS-9665</td>
<td>—</td>
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<tr>
<td>Clear shell, barcoded</td>
<td>HSS-9901</td>
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<tr>
<td>Hard-Shell 384-Well Standard PCR Plates (50 plates)</td>
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<tr>
<td>Clear shell</td>
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<tr>
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<tr>
<td>Blue shell</td>
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<td>HSP-3841</td>
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<td>HSP-3865</td>
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<table>
<thead>
<tr>
<th>Description</th>
<th>Clear Well</th>
<th>White Well</th>
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<td>Hard-Shell 96-Well 480 PCR Plates</td>
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<td></td>
</tr>
<tr>
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<tr>
<td>Clear shell, white well</td>
<td>HSR-9905</td>
<td>HSR-9005K</td>
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<tr>
<td>Clear shell, clear well</td>
<td>HSR-9901</td>
<td>HSR-9901K</td>
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<table>
<thead>
<tr>
<th>Description</th>
<th>Clear Well</th>
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<td>Hard-Shell 384-Well 480 PCR Plates</td>
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<td>Clear shell, white well</td>
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<td>HSR-4805K</td>
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<tr>
<td>Clear shell, clear well</td>
<td>HSR-4801</td>
<td>HSR-4801K</td>
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</table>
PrimePCR™ ASSAYS AND PANELS

PrimePCR Assays and Panels for real-time PCR are expertly designed and wet-lab validated to ensure optimal assay performance and compliance.

- Expertly designed PCR primer and probe assays for qPCR, preamplification, and Droplet Digital™ PCR
- Wet-lab validated for guaranteed performance; each assay for the human, mouse, and rat genomes was experimentally tested for optimal efficiency, specificity, sensitivity, and linear dynamic range
- Assays for gene expression analysis, copy number variation, and mutation detection
- Compliant with the MIQE guidelines
Why PrimePCR?

- Offers guaranteed performance
- Eliminates time-consuming optimization
- Aids in MIQE compliance

Wide Range of Predesigned Disease- and Pathway-Specific Panels

- Expertly curated to include the most biologically relevant gene targets
- Customizable to include different gene targets of interest
- Integrated with CFX Manager™ Software

Complete Solution for Real-Time PCR

- Aurum™ Total RNA Kits
- iScript™ cDNA Synthesis Kits
- Supermixes for qPCR
- SsoAdvanced™ PreAmp Supermix and PrimePCR PreAmp Assays
- PCR plates and tubes
- Real-time PCR instruments
- CFX Manager Software
- PrimePCR Analysis Software

Assay Design

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Assay specificity confirmed by next-generation sequencing</th>
<th>Avoided common single nucleotide polymorphisms in target regions</th>
<th>Designed intron-spanning assays whenever possible</th>
<th>Avoided secondary structures in primer annealing sites</th>
<th>Maximized fraction of transcript isoforms being detected</th>
<th>Compatible with standard assay conditions</th>
</tr>
</thead>
</table>

* Human, mouse, and rat genomes.
PrimePCR Real-Time PCR Products

**Predesigned SYBR® Green Assays**
Transcriptome-wide primer assays for SYBR® Green gene expression analysis are available in 200, 1,000, or 2,500 reaction sizes.

**Predesigned Probe Assays**
Transcriptome-wide probe assays for gene expression analysis are available in 500, 1,000, or 2,500 reaction sizes.

**Custom Assays**
User-defined primer and probe sequences can be ordered.

**PreAmp Assays**
Primers are available for target-specific preamplification of limited nucleic acid.

**DNA Templates**
Gene-specific synthetic DNA templates are designed to give a positive real-time PCR result when used with the corresponding gene assay.

**Experimental Controls**
Control assays can be used to assess reverse transcription, RNA quality, gDNA contamination, and PCR performance.

**Predesigned Pathway Panels**
A large selection of predesigned disease- and pathway-specific panels are available.

**Custom PCR Plates**
Custom-configured 96- and 384-well PCR plates can be ordered with SYBR® Green assays.
PrimePCR Assay Selection Guide and Lookup Tool

PrimePCR Assay Selection Guide
PrimePCR assays are available for real-time PCR or Droplet Digital™ PCR.

<table>
<thead>
<tr>
<th>Real-Time PCR Assays (wet-lab validated)</th>
<th>Droplet Digital PCR Assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organisms</td>
<td>Human</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
</tr>
<tr>
<td>Assay chemistry</td>
<td>SYBR® Green and probe assays</td>
</tr>
<tr>
<td></td>
<td>Probe assays</td>
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<td>Applications</td>
<td>Gene expression</td>
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<td>Preamplification</td>
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<td>Copy number variation</td>
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<td>Mutation detection</td>
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<tr>
<td>Formats</td>
<td>Individual assays</td>
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<tr>
<td></td>
<td>96- and 384-well predesigned plates*</td>
</tr>
<tr>
<td></td>
<td>96- and 384-well custom plates*</td>
</tr>
</tbody>
</table>

* Probe assays are not available in plates.

PrimePCR Lookup Tool
Use the PrimePCR Lookup Tool to find assays and panels for your genes of interest.

For more information, download or request bulletins 6290, 6512, and 6595.
PrimePCR™ ASSAYS AND PANELS
Assay Performance Standards and Validation

Assay Performance Standards and Validation

<table>
<thead>
<tr>
<th>Standard</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>Accurate detection of 20 copies</td>
</tr>
<tr>
<td>Specificity</td>
<td>Validated amplicon sequence with next-generation sequencing; minimal primer-dimer formation and gDNA cross-reactivity</td>
</tr>
<tr>
<td>Amplification efficiency</td>
<td>90–110%</td>
</tr>
<tr>
<td>Linear dynamic range</td>
<td>Minimum of 6 orders of magnitude; detection of a synthetic template standard curve from 20 to 20,000,000 copies</td>
</tr>
<tr>
<td>$R^2$</td>
<td>&gt;0.98</td>
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</table>

Assay Validation
PrimePCR Assays for the human, mouse, and rat genomes were validated using iScript™ Advanced cDNA Synthesis Kit for RT-qPCR and SsoAdvanced™ SYBR® Green Supermix on an automated CFX384 Touch™ Real-Time PCR Detection System.
Predesigned Pathway Panels

The Most Focused Approach for Real-Time PCR
Bio-Rad collaborated with Thomson Reuters to expertly design an extensive range of predesigned panels. Each real-time PCR plate contains the most biologically relevant gene targets in a canonical pathway, disease, or biological process.

- Obtain complete pathway data from a single experiment
- Visualize biological interactions using interactive pathway maps
- Gain new insights using integrated data analysis tools

Pathway Panels
Pathway-Focused Analysis
PrimePCR Pathway Panels were designed for more than 300 canonical pathways. Pathway panels enable complete pathway analysis for differentially expressed, top-ranked gene targets.

Collection Panels
Broad Target Exploration
PrimePCR Collection Panels represent the top-ranked gene targets for differential gene expression analysis, allowing for a more general survey of gene targets across a biological process or group.

Disease Panels
Disease-Focused Analysis
PrimePCR Disease State Panels were designed by referencing the National Library of Medicine Medical Subject Headings (MeSH) database. Disease state panels allow for the thorough investigation of previously published, differentially expressed genes within a specified pathology.

Target Ranking
Gene assays present on predesigned panels have been prioritized based on three main criteria:
- How often a gene changes expression level in transcriptome studies
- How much attention was paid to this gene in the overall scientific research
- How interesting the scientific community found this gene in the last 2 years
PrimePCR™ ASSAYS AND PANELS
PrimePCR Panels for a Broad Range of Pathways and Disease States

Diseases
- Bacterial infections and fungal mycoses
- Cancer and neoplasms
- Cardiac hypertrophy
- Cardiovascular diseases
- Congenital, hereditary, and neonatal diseases and abnormalities
- Cystic fibrosis
- Digestive system diseases
- Endocrine system diseases
- Eye diseases
- Female urogenital diseases and pregnancy complications
- Hemic and lymphatic diseases
- Immune system diseases
- Male urogenital diseases
- Mental disorders
- Musculoskeletal diseases
- Nervous system diseases
- Nutritional and metabolic diseases
- Otorhinolaryngologic diseases
- Parasitic diseases
- Pathological conditions, signs, and symptoms
- Respiratory tract diseases
- Skin and connective tissue diseases
- Stomatognathic diseases
- Viral diseases
- Wounds and injuries

Processes
- Apoptosis and survival
- Blood coagulation
- Cell adhesion
- Cell cycle
- Chemotaxis
- Cytoskeleton remodeling
- Development
- DNA damage
- Hypoxia response
- Immune response
- Muscle contraction
- Neurophysiological process
- Oxidative stress
- Proteolysis
- Reproduction
- Transcription
- Translation
- Transport

Metabolism
- Amino acid metabolism
- Carbohydrate metabolism
- Lipid metabolism
- Nucleotide metabolism
- Regulation of lipid metabolism
- Regulation of metabolism
- Steroid metabolism
- Vitamin and cofactor metabolism
- Xenobiotic metabolism

Protein Function
- Cytokines and chemokines
- G proteins
- Growth factors
- Hormones
- Kinases
- Phosphatases
- Second messengers
- Transcription factors

More than 1,000 unique panels are available in these categories. Visit bio-rad.com/web/PrimePCRpanels for more information.
Custom PCR Plates

Design a custom plate with PrimePCR Assays using 96-well or 384-well plates, which are available for every major real-time PCR instrument. Customize your plate design layout or use a suggested plate template as a guide, then select PrimePCR Assays or add your own custom assays.

1. Visit bio-rad.com/PrimePCR and click Create a Custom PCR Plate button.

2. Select a plate template.

3. Search and select PrimePCR Assays.

4. Lay out assays, controls, and references.

5. Review configuration. Plate configurations are automatically saved to your My PrimePCR hot list for easy modifications or reorder.
## Experimental Controls and Reference Gene Assays

### Controls

Experimental control assays and synthetic DNA templates are designed to assess the key experimental factors that may impact your real-time PCR results.

<p>| | | | | | | | | | | | | |</p>
<table>
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<td>64</td>
<td>72</td>
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<td>RT</td>
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</tbody>
</table>

### Reference Gene Assays

We have suggested a set of commonly used reference genes that can be used individually, or easily screened using our preplated 96-well and 384-well reference gene panels. Reference gene assays may also be added to custom-designed plates.

#### DNA Contamination Control Assay

The PrimePCR DNA Contamination Control Assay is designed to determine if gDNA is present in a sample at a level that may affect PCR results.

#### Positive PCR Control Assay

The PrimePCR Positive Control Assay is designed to assess how a given experimental sample may adversely affect PCR performance.

#### RNA Quality Assay

The PrimePCR RNA Quality Assay is designed to determine whether RNA integrity may adversely affect PCR results.

#### Reverse Transcription Control Assay

The PrimePCR Reverse Transcription Control Assay is designed to qualitatively assess the performance of the RT reaction.
PrimePCR Data Analysis

CFX Manager™ Software
Ordering a PrimePCR Plate or Assay is just the beginning of a seamless and integrated workflow, from reaction setup to data acquisition and analysis using CFX Manager Software and PrimePCR Analysis Software. The expert design and wet-lab validation of PrimePCR Assays for the human, mouse, and rat genomes ensure optimal assay performance so that time once spent optimizing runs can now be devoted to analyzing and interpreting experimental results.

- Select PrimePCR and start a run with a single click
- Begin analyzing data in a single step by importing target, reference, and control information from a PrimePCR run file directly into the plate layout
- Combine data from multiple plates into a Gene Study to rapidly screen large numbers of targets or samples
- Use powerful data visualization tools, such as hierarchical clustering and color-matched expression levels, to identify individual targets or clusters to consider for further investigation

PrimePCR Runs from Start to Finish
Start runs quickly by choosing PrimePCR in the Startup Wizard to select the validated PrimePCR run protocol, then click Start Run. CFX Manager Software is fully integrated with PrimePCR products for a fast, streamlined path from data generation to data analysis.

PrimePCR™ Analysis Software
For non-CFX platforms, Bio-Rad offers a PrimePCR data analysis solution. Visit bio-rad.com/PrimePCR to download the software. Easily upload Cq values and quickly generate meaningful information from your gene expression experiment.
PCR RUN AND DATA ANALYSIS

Bio-Rad’s real-time PCR systems are designed to deliver outstanding thermal performance and sensitive optical detection. By pairing proven real-time instruments with quality PCR reagents and powerful analysis software, you can consistently obtain high-quality data to fuel your research discoveries.

- CFX Real-Time PCR Detection Systems efficiently provide accurate, reliable data
- CFX Manager™ Software enables the collection and analysis of real-time data
- PrimePCR™ Analysis Software is a stand-alone data analysis tool for non-Bio-Rad instruments
Overview of CFX Systems and Software

CFX Real-Time PCR Detection Systems
Bio-Rad’s real-time PCR systems combine a thermal cycler and optical reaction module for singleplex and multiplex detection of fluorophores. The systems feature thermal gradient functionality and automation capabilities.

- CFX96 Touch™ Real-Time PCR Detection System
- CFX96 Touch Deep Well Real-Time PCR Detection System
- CFX384 Touch™ Real-Time PCR Detection System
- CFX Connect™ Real-Time PCR Detection System

CFX Manager™ Software
CFX Manager Software accommodates individual user needs and different types of experiments with intuitive navigation and customizable settings.

With CFX Manager Software you can:

- **Get started quickly** — use intuitive navigation, a new Startup Wizard, and a streamlined interface
- **Stay organized** — reserve multiple instruments using the Scheduler and rapidly set up reactions with the Master Mix Calculator
- **Analyze results when and where you want** — receive email notification with an attached data file when a run is finished
- **Make decisions about your data faster** — visualize all of your run’s data easily with Custom Data View

- **Extract more meaningful information from your run** — analyze data using bar chart, clustergram, scatter plot, volcano plot, or heat map analysis employing multiple reference genes and individual reaction efficiencies
- **Export only the data you want** — specify what to export and the preferred format with Custom Data Export

Easily identify specific samples using the multipane data highlighting feature in CFX Manager Software.

PrimePCR™ Analysis Software
For non-CFX platforms, Bio-Rad offers a PrimePCR data analysis solution. Visit [bio-rad.com/web/PrimePCRsoftware](http://bio-rad.com/web/PrimePCRsoftware) to download the software. Easily upload Cq values and quickly generate meaningful information from your gene expression experiment.
Reference


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Purchase of iProof High-Fidelity DNA Polymerase or iTaq DNA Polymerase 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. No other patent rights are conveyed expressly, by implication, or by estoppel. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

The use of iQ, iTaq, SsoAdvanced, and SsoFast Supermixes and PrimePCR PreAmp Assays is covered by one or more of the following U.S. patents and corresponding patent claims outside the U.S.: 5,804,375; 5,538,848; 5,723,591; 5,876,930; 5,994,056; 6,030,787; 6,171,785; and 6,258,569. The purchase of these products includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser’s own internal research. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser’s activities for a fee or other commercial consideration, are conveyed expressly, by implication, or by estoppel. These products are for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained from the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

Hard-Shell Plates are covered by one or more of the following U.S. patents or their foreign counterparts owned by Eppendorf AG: U.S. Patent Numbers 7,347,977; 6,340,589; and 6,528,302.

Visit bio-rad.com/web/AmpConsumables for more information.