

Amplification Reagents and Plastics



PCR AND GENE EXPRESSION WORKFLOW



1

Pages 2-5

- SingleShot Cell Lysis RT-qPCR Kits
- One-Step and Two-Step
 SYBR® Green or Probes Formats



2

Pages 6-9

- RNA Isolation Kits Selection Guide
- Aurum Sample Preparation Kits and PureZOL RNA Isolation Reagent
- Ordering Information



3

Pages 10-15

- Reverse Transcription Kit Selector
- Reliance Select cDNA Synthesis Kit
- iScript cDNA Synthesis Kits
- Ordering Information



PCR AND REAL-TIME PCR REAGENTS

4

Pages 16-35

- Real-Time PCR Reagents Selection Guide
- SsoAdvanced Universal Supermixes
- iTaq Universal Supermixes
- Reliance One-Step Multiplex RT-qPCR Supermix
- iTaq Universal One-Step Kits
- iQ Supermixes
- Precision Melt Supermix
- Precision Blue Real-Time PCR Dye
- High-Fidelity and Standard PCR Reagents
- Real-Time PCR Reagent Compatibility with Instruments
- Ordering Information



5 Pages 36–39

- PreamplificationSelection Guide
- iScript Explore
 One-Step RT and
 PreAmp Kit
- SsoAdvancedPreAmp Supermix
- Ordering Information



PCR PLASTIC CONSUMABLES

6

Pages 40-49

- Instrument Compatibility
- PCR Tubes and Strips
- Hard-Shell PCR Plates
- PCR Plates
- PCR Plate Sealing
- Ordering Information



PrimePCR ASSAYS AND PANELS

7

Pages 50-59

- High-Performance Assay Solutions
- PrimePCR Real-Time PCR Products
- Assay Performance Standards and Validation
- Predesigned mRNA Pathway Panels
- PrimePCR Panels for a Broad Range of Pathways and Disease States
- Predesigned and Custom PrimePCR Long Noncoding RNA (IncRNA) Arrays
- PrimePCR Long Noncoding RNA Assays
- Experimental Controls and Reference Gene Assays
- PrimePCR Data Analysis



PCR RUN AND DATA ANALYSIS

8

Pages 60-61

 Overview of CFX Systems and Software

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 up to 100,000 cells in 20 minutes
- Simple protocol for automated, high-throughput reverse transcription qPCR (RT-qPCR) experiments
- Available in one- or two-step SYBR® Green or probes kits

SingleShot Cell Lysis RT-qPCR Kits

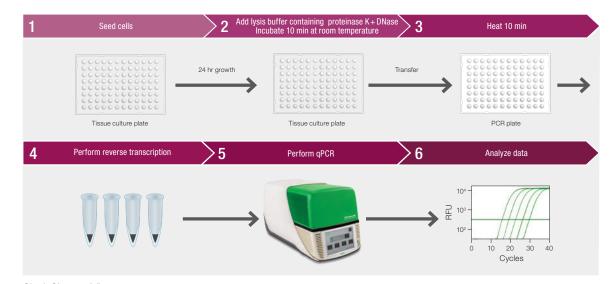
All SingleShot Cell Lysis RT-qPCR Kits feature:

- 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 to be compatible with PrimePCR Assays and Panels in preamplification workflows using 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.

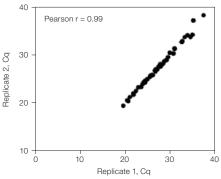
| SingleShot Kit | Features | Product Options |
|----------------------------------|--|---|
| Cell Lysis Kits | Cell lysis for RT-qPCRRT-qPCR reagents sold separatelyNo RNA purification needed | 100 reactions, catalog #1725080 500 reactions, catalog #1725081 |
| Cell Lysis Two-Step RT-qPCR Kits | Cell 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 #1725085 Probes, 100 reactions, catalog #1725090 |
| 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 #1725095 Probes, 100 reactions, catalog #1725070 |

One-Step and Two-Step SYBR® Green or Probes Formats

SingleShot Cell Lysis Kits

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

Reproducibility of Cell Lysates



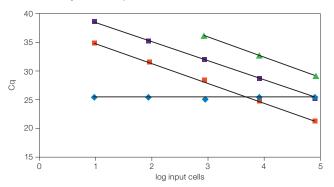
The SingleShot SYBR® Green Kit demonstrates high reproducibility between technical lysate replicates. Two technical lysate replicates of four neuroblastoma cell lines (SH-EP, SK-N-AS, NGP, and IMR-32) were examined using ten SYBR® Green qPCR Assays to evaluate the reproducibility of the SingleShot SYBR® 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



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 to 100,000 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

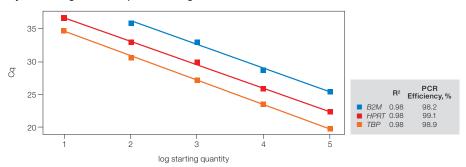
SingleShot Cell Lysis One-Step RT-qPCR Kits

- Within 1.5 hr after cell lysis, yields high-performance RT-qPCR data directly from cell culture lysates
- Cell lysate up 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



Visit bio-rad.com/SingleShot or download or request bulletins 6572 and 6604 for more information.

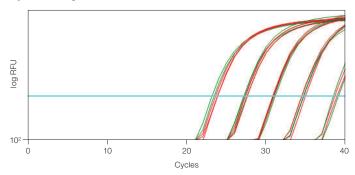
Dynamic Range across Expression Ranges



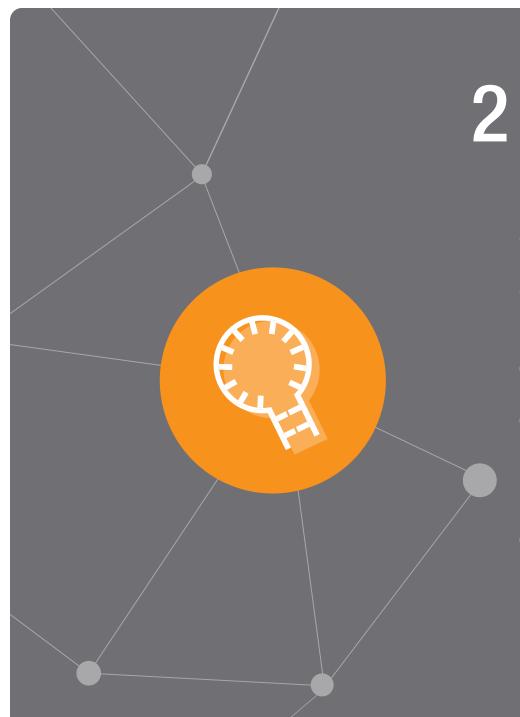
SingleShot SYBR® Green One-Step Kit enables a large dynamic range.

Two technical lysate replicates were examined across three differentially expressed genes (*B2M*, *HPRT*, *TBP*) using a human kidney tumor (HKT) cell line to evaluate the linear dynamic range of the SingleShot SYBR® Green One-Step Kit. The kit showed a large dynamic range across all assayed expression levels. Cq, quantification cycle.

Dynamic Range Similar to Purified RNA



SingleShot SYBR® Green One-Step Kit lysates yield a dynamic range similar to that of purified RNA. Gene expression data generated using SingleShot SYBR® Green One-Step Kit (■) lysates and isolated RNA (Aurum Total RNA Mini Kit, ■) were compared using a human cell line (K652) and from 10 to 100,000 input cells. B2M expression was assessed using a PrimePCR qPCR Assay. SingleShot SYBR® Green One-Step Kit lysates showed a dynamic range similar to that of the RNA generated using the Aurum Total RNA Mini Kit without the need for RNA isolation. RFU, relative fluorescence units.



2 RNA ISOLATION

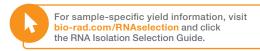
Bio-Rad provides two distinct technologies for isolating total RNA. Silica membrane—based kits, available in 96-well plate and spin column formats, and PureZOL RNA Isolation Reagent, which is ideal for scaling up isolation protocols.

- 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
 - PCR or real-time PCR
 - Northern blotting
 - Microarray analysis
 - cDNA library construction
- DNase treatment ensures gDNA removal

RNA Isolation Kits Selection Guide

| | Aurum Total RNA Kits | | | |
|---------------------------------------|---|---|---|--|
| | Mini | Fatty and Fibrous Tissue | 96 | PureZOL RNA Isolation Reagent |
| Description | Guanidine isothiocyanate and β-mercaptoethanol efficiently lyse samples and quickly inactivate RNases | 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 | Kit is composed of guanidine isothiocyanate and β-mercaptoethanol for efficient lysis and quick RNase inactivation, followed by purification in a 96-well plate | PureZOL efficiently lyses cells and tissues, deproteinates RNA, and inactivates endogenous nucleases in a single step |
| Format | Mini column Filtration (vacuum or spin) | Mini column Filtration (vacuum or spin) | 96-well plate Filtration (vacuum or spin) | Single solution organic extraction |
| Maximum starting material amounts | | | | |
| Cultured cells | 2 x 10 ⁶ | 1 x 10 ⁷ | 1 x 10 ⁶ | 1 x 10 ⁷ |
| Bacterial cells | 2.4 x 10 ⁹ | 2.4 x 10 ⁹ | 8 x 10 ⁸ | 2.4 x 10 ⁹ |
| Yeast cells | 3 x 10 ⁷ | 3 x 10 ⁷ | 2 x 10 ⁷ | 3 x 10 ⁷ |
| Hard animal tissue | 20 mg | 100 mg | _ | 100 mg |
| Soft to moderately hard animal tissue | 40 mg | 100 mg | - | 100 mg |
| Plant tissue | 40 mg | 100 mg | _ | 100 mg |
| Isolation method | Silica membrane | Lysis with PureZOL reagent, purification on silica membrane | Silica membrane | Organic extraction |
| Number of preps | 50 mini preps | 50 mini preps | 2 x 96-well plate | 50 or 100 (1 ml/prep) |
| Number of washes | 3 | 3 | 3 | _ |
| DNase I included* | Yes | Yes | Yes | No |
| DNase I digest time | 15 min (animal tissue, 25 min) | 15 min | 10 min | _ |
| Total preparation time** | <50-80 min (with DNase I digest) | <50-80 min (with DNase I digest) | <60 min (with DNase I digest) | <60 min |
| Binding capacity | >100 µg | >100 µg | >40 µg | _ |
| Elution volume | 2 x 40 µl | 2 x 40 µl | 80 µl | 30–100 μl |

^{*} Removal not required.



^{**} Total preparation time will vary depending on the tissue or cell type and which format is used (vacuum or spin).

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

7326820, 50 preps Aurum Total RNA Mini 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



7326830, 50 preps Aurum Total RNA Fatty and Fibrous Tissue Kit



7326800, 2 x 96-well preps Aurum Total RNA 96 Kit



7326890, 100 ml PureZOL RNA Isolation Reagent



Ordering Information

| Catalog # | Description |
|-----------|---|
| 7326830 | Aurum Total RNA Fatty and Fibrous Tissue Kit |
| 7326870* | Aurum Total RNA Fatty and Fibrous Tissue Module |
| 7326820 | Aurum Total RNA Mini Kit |
| 7326800 | Aurum Total RNA 96 Kit |
| 7326880 | PureZOL RNA Isolation Reagent, 50 ml |
| 7326890 | PureZOL RNA Isolation Reagent, 100 ml |

^{*} Not provided with PureZOL RNA Isolation Reagent (see catalog #7326880 or #7326890 to order separately).

3 REVERSE TRANSCRIPTION

Bio-Rad reverse transcription kits provide easy setup and high reproducibility for first-strand cDNA synthesis. Bio-Rad 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



REVERSE TRANSCRIPTION Reverse Transcription Kit Selector

Reverse Transcription Kit Selector













| | Reliance Select cDNA Synthesis Kit | iScript Reverse Transcription Supermix for RT-qPCR | iScript gDNA Clear cDNA Synthesis Kit | iScript Advanced cDNA Synthesis Kit for RT-qPCR | iScript Select cDNA Synthesis Kit | iScript cDNA Synthesis Kit |
|------------------------------------|--|--|--|--|---|--|
| Features | Overcome challenging samples | Minimum reaction setup time | Effective gDNA removal before reverse transcription | Maximum input RNA for high cDNA yields | Flexible priming options and high fidelity | Reliable value solution |
| Applications | Cloning Sequencing and next- generation sequencing Gene expression RNA quantification | Gene expressionRNA quantification | Gene expressionRNA quantification | Gene expressionRNA quantification | Cloning Sequencing and next-generation sequencing Gene expression RNA quantification | Gene expressionRNA quantification |
| Total Input RNA Range | 2 μg–100 fg | 1 μg–1 pg | 1 µg–1 pg | 7.5 μg–100 fg | 1 μg–1 pg | 1 μg-100 fg |
| Kit Format | 6 tubes | 1 tube | 3 tubes | 2 tubes | 5 tubes | 2 tubes |
| Priming Options | Random primersOligo(dT)Gene specific | Blend of random primers and oligo(dT) | Blend of random primers and oligo(dT) | Blend of random primers and oligo(dT) | Random primersOligo(dT)Gene specific | Blend of random primers and oligo(dT) |
| Optimal Reaction Temperature | 50°C | 46°C | 46°C | 46°C | 42°C | 46°C |
| DNase Step Included | V | | ~ | | | |
| Time to Produce cDNA | 10 min | 26 min | 36 min | 21 min | 15–65 min | 26 min |
| RNase H+ Activity | Reduced | ~ | V | V | V | V |



Go to bio-rad.com/PCRReagentSelector and use our interactive selection tool to find the right reverse transcriptase for your needs.

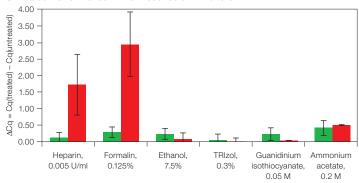
3 REVERSE TRANSCRIPTION Reliance Select cDNA Synthesis Kit

Reliance Select cDNA Synthesis Kit

The Reliance Select cDNA Synthesis Kit is a high-performance product featuring Reliance Reverse Transcriptase, a novel chimeric enzyme that delivers superior results across a broad dynamic range, even with challenging samples.

- Create cDNA libraries from a wide range of inputs and a variety of sample types
- Overcome reaction inhibitors
- Power through complex secondary structures and GC-rich regions (up to 80%)
- Eliminate gDNA for more accurate RT-qPCR
- Shorten time to results
- Optimize experimental design with flexible priming strategies

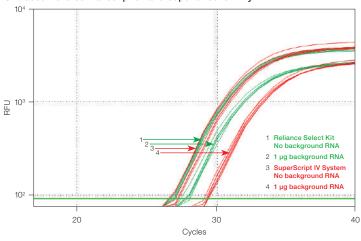
Unrivaled Performance in the Presence of Inhibitors



The Reliance Select cDNA Synthesis Kit demonstrates superior efficiency compared to the SuperScript IV First-Strand Synthesis System (Thermo Fisher Scientific Inc.) in the presence of inhibitors. The Reliance Select Kit (\blacksquare) delivers more consistent results and earlier average Cq values than the SuperScript IV System (\blacksquare). This is evident from the lower Δ Cq values of treated versus untreated reactions.

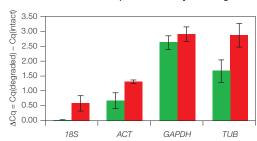
Reliance Select cDNA Synthesis Kit

Unbiased Reverse Transcription and Superior Sensitivity



The Reliance Select cDNA Synthesis Kit allows for sensitive detection of low-abundance transcripts. The Reliance Select Kit allows for more sensitive detection of External RNA Controls Consortium (ERCC) spike-in RNAs in the presence of background RNA compared to the SuperScript IV System. RFU, relative fluorescence units.

Greater Reverse Transcription Efficiency with Degraded Samples



The Reliance Select cDNA Synthesis Kit delivers earlier Cq values compared to the SuperScript IV System when tested with artificially degraded RNA samples. The Reliance Select Kit (■) delivers more accurate results between degraded and intact RNAs than the SuperScript IV System (■). This is evident from the lower Δ Cq values of degraded versus intact reactions.

Ordering Information

| Catalog # | Description |
|-----------|---|
| 12012802 | Reliance Select cDNA Synthesis Kit, 25 x 20 µl reactions |
| 12012801 | Reliance Select cDNA Synthesis Kit, $100 \times 20 \mu l$ reactions |
| 17006111 | Reliance Select cDNA Synthesis Kit, 500 x 20 µl reactions |



3 REVERSE TRANSCRIPTION iScript cDNA Synthesis Kits

iScript cDNA Synthesis Kits

iScript Performance

iScript Reverse Transcription Kits were designed for easy setup, high reproducibility, and bias-free cDNA synthesis. iScript reagent features include:

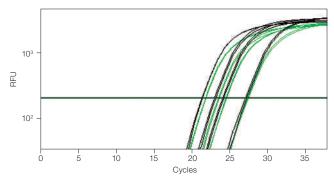
- RNase H+ activity of the reverse transcriptase ensures 1:1 RNA:cDNA conversion, resulting in greater sensitivity and more accurate data
- Optimal blend of oligo(dT) and random primers for complete and unbiased coverage
- Potent blend of RNase inhibitors prevents RNA degradation during reaction setup and cDNA synthesis steps



Watch the tutorial!
Understanding Reverse Transcription
bio-rad.com/RT-tutorial

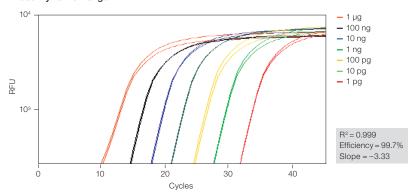


Superior 5' to 3' Coverage



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 System. Primer pairs were designed at the 5' end (■, 91 bp amplicon) and 3' end (■, 69 bp amplicon). 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.

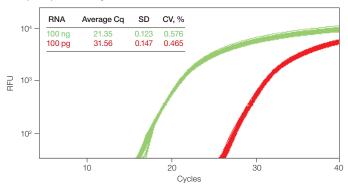
Broad Dynamic Range



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 the β-actin gene (~90 bp) in 20 μl qPCR reactions with iTaq Universal SYBR® Green Supermix. RFU, relative fluorescence units.

iScript cDNA Synthesis Kits

iScript Reproducibility

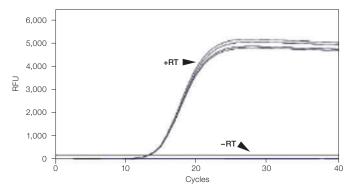


Excellent data reproducibility. PGK-1 mRNA (~160 bp), a gene that encodes a glycolytic enzyme, was quantified using iScript Reverse Transcription Supermix for RT-qPCR with both 100 ng (■) and 100 pg (II) of input RNA. For each input RNA, 48 individual RT reactions were performed and one-tenth of the resulting cDNA was used in the gPCR reaction with SsoFast Probes Supermix. The gene expression analysis data show excellent reproducibility with both 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.

Ordering Information

| Catalog # | Description |
|--------------|--|
| cDNA Synthes | sis Reagents |
| 1725037 | iScript Advanced cDNA Synthesis Kit for RT-qPCR, 25 x 20 µl reactions |
| 1725038 | iScript Advanced cDNA Synthesis Kit for RT-qPCR, $100 \times 20 \mu l$ reactions |
| 1725038BUN | iScript Advanced cDNA Synthesis Kit for RT-qPCR, $500 \times 20 \mu l$ reactions |
| 1708890 | iScript cDNA Synthesis Kit, 25 x 20 µl reactions |
| 1708891 | iScript cDNA Synthesis Kit, 100 x 20 μl reactions |
| 1725034 | iScript gDNA Clear cDNA Synthesis Kit, $25 \times 20 \mu l$ reactions |
| 1725035 | iScript gDNA Clear cDNA Synthesis Kit, $100 \times 20 \mu l$ reactions |
| 1725035BUN | iScript gDNA Clear cDNA Synthesis Kit, $500 \times 20 \ \mu l$ reactions |

Effective Removal of Genomic DNA



absence of amplification in the -RT sample. RFU, relative fluorescence units.

iScript gDNA Clear cDNA Synthesis Kit eliminates contaminating genomic DNA. HeLa RNA (1 µg) was spiked in with 300 ng gDNA. Contaminating gDNA was cleared and cDNA synthesis was then performed with (+RT) and without (-RT) reverse transcriptase using iScript qDNA Clear cDNA Synthesis Kit. qPCR was performed using the human TUBA assay, an exonic assay that detects both mRNA and gDNA. Complete removal of gDNA is demonstrated by the

| Catalog # | Description |
|------------|---|
| 1708840 | iScript Reverse Transcription Supermix for RT-qPCR, $25 \times 20 \mu l$ reactions |
| 1708841 | iScript Reverse Transcription Supermix for RT-qPCR, 100 x 20 μ l reactions |
| 1708841BUN | iScript Reverse Transcription Supermix for RT-qPCR, $500 \times 20 \ \mu l$ reactions |
| 1708896 | iScript Select cDNA Synthesis Kit, $25 \times 20 \mu l$ reactions |
| 1708897 | iScript Select cDNA Synthesis Kit, $100 \times 20 \ \mu l$ reactions |

4 PCR AND REAL-TIME PCR REAGENTS

Bio-Rad PCR and real-time PCR reagents and kits feature advanced enzyme technology and buffer formulation for robust amplification and detection with maximum sensitivity, specificity, and efficiency.

- Antibody-mediated hot-start technology enables instant polymerase activation and superior specificity
- Patented Sso7d fusion enzyme technology delivers higher processivity and inhibitor tolerance
- Faster time to results without compromising qPCR data quality
- High performance obtained on any instrument, under any reaction and/or cycling conditions
- A novel chimeric Reliance Reverse Transcriptase delivers superior one-step RT-qPCR results, even with challenging samples

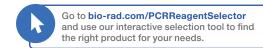


Real-Time PCR Reagents Selection Guide

Use this selection guide to choose an appropriate reagent for your application.

| | qPCR (DNA to Cq) | | | One-Step RT-q | One-Step RT-qPCR (RNA to Cq) | |
|--|--|---|---|--|---|--|
| | SsoAdvanced Universal Supermixes | SsoAdvanced Universal Inhibitor-Tolerant Supermix | iTaq Universal Supermixes | Reliance One-Step Multiplex Supermix | iTaq Universal One-Step Kits | |
| Features | Robust Sso7d-driven performance with demanding templates and under challenging conditions | Sso7d-driven optimal performance for samples containing PCR inhibitors | Advanced formulation for reliable, reproducible performance | Single-tube, 4x supermix for 5-plex reaction with 24 hr assembled reaction stability | Easy 2-tube system for SYBR® Green– and probe-based one- step RT-qPCR | |
| Sensitive detection of low-abundance targets | •••• | •••• | •••• | •••• | •••• | |
| Efficient amplification of difficult targets | •••• | •••• | ••• | •••• | ••• | |
| Specific amplification of targets | •••• | •••• | •••• | •••• | •••• | |
| Robust performance under varying PCR conditions* | •••• | •••• | ••• | ••• | ••• | |
| Tolerance to PCR inhibitors | •••• | •••• | ••• | •••• | ••• | |
| Fast cycling compatibility | Yes | Yes | Yes | Yes | Yes | |
| Available detection chemistry | SYBR® Green and probes | SYBR® Green | SYBR® Green and probes | Probes | SYBR® Green and probes | |
| Compatibility with any qPCR instrument | Any instrument | Any instrument | Any instrument | Any instrument | Any instrument | |
| Samples | Blood spot Plant extract Cell lysate Plant tissue Crude Plasmid extraction Purified FFPE gDNA and cDNA | Blood spot Plant extract Cell lysate Plant tissue Crude Plasmid extraction Purified gDNA and cDNA | PlasmidPurified gDNA and cDNA | Cell lysate up to 10 μlPurified RNA | ■ Cell Iysate up to 4 µI ■ Purified RNA | |

^{•••} Fair •••• Good •••• Excellent



cDNA, complementary DNA; Cq, quantification cycle; FFPE, formalin-fixed paraffin-embedded; gDNA, genomic DNA; LCM, laser capture microdissection; RT-qPCR, reverse transcription quantitative PCR.

^{*} Primer concentrations, annealing temperatures, salt concentrations, and/or cycling protocol (standard or fast).

4 PCR AND REAL-TIME PCR REAGENTS SsoAdvanced Universal Supermixes

SsoAdvanced Universal Supermixes

Innovative Sso7d Fusion Enzyme Technology for Robust qPCR

SsoAdvanced Universal Supermixes are exclusive high-performance real-time PCR reagents based on the Bio-Rad patented* Sso7d fusion protein polymerase technology and advanced buffer formulation.

The supermixes are uniquely formulated to provide higher processivity, increased PCR inhibitor tolerance, and robust performance with challenging templates and targets in SYBR® Green– or probe-based qPCR. They deliver superior performance for a wide range of applications on any qPCR platform and are validated to ensure optimal results with Bio-Rad PrimePCR Gene Expression Assays.

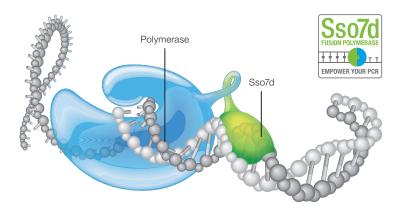
SsoAdvanced Universal Supermixes allow you to:

- Achieve superior real-time PCR results under various experimental conditions — our robust formulation delivers consistent performance in fast and standard cycling across a broad range of reaction conditions, primer concentrations, and temperature ranges
- Increase qPCR sensitivity and efficiency of detection from compromised samples — the highly processive Sso7d fusion polymerase exhibits increased resistance to a wide variety of PCR inhibitors, enhancing sensitivity and overall performance
- Shorten time to results while maintaining qPCR data quality the Sso7d fusion polymerase and optimized buffer provide rapid polymerization kinetics and instant polymerase activation
- 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

 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

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.

Watch the tutorial! Understanding Real-Time PCR Supermixes bio-rad.com/RTsmxTutorial



Visit bio-rad.com/Supermixes or download or request bulletin 7178 for more information.

18S rRNA efficiency = 98% TFRC efficiency = 101%

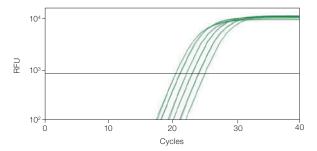
SsoAdvanced Universal Supermixes

SsoAdvanced Universal SYBR® Green and Probes Supermixes

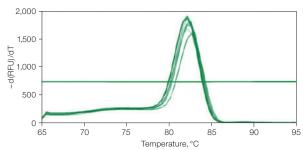
SsoAdvanced Universal Supermixes employing our patented* Sso7d fusion enzyme technology provide higher processivity, increased PCR inhibitor tolerance, and robust performance with challenging templates and target sequences in real-time PCR.

SsoAdvanced Universal SYBR® Green Supermix

A. Optimal PCR Sensitivity



B. Superior PCR Specificity

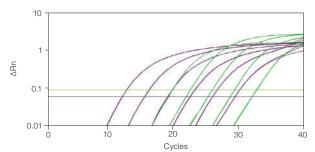


Optimal PCR sensitivity and specificity with SsoAdvanced Universal SYBR® Green Supermix. A, a PrimePCR Assay targeting β_o microglobulin, using 250, 125, 62.5, 31.25, 15.625, and 7.8125 cells/20 µl reaction, was performed on a CFX96 Real-Time PCR System and yielded optimal PCR sensitivity; B, melt curve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

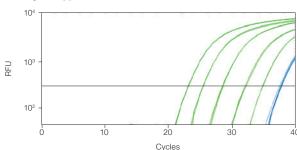


SsoAdvanced Universal Probes Supermix

A. Superior Multiplex qPCR Performance



B. Single-Copy Detection



Superior qPCR performance in a duplex reaction (A) and single-copy detection (B) with SsoAdvanced Universal Probes Supermix. A, predesigned TagMan 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 System. RFU, relative fluorescence units; ΔRn , baseline-corrected normalized reporter.

^{*} U.S. patents 6,627,424; 7,541,170; and 7,560,260.

4 PCR AND REAL-TIME PCR REAGENTS SsoAdvanced Universal Supermixes

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.

- Power through PCR inhibitors delivers unrivaled performance with common PCR inhibitors, such as heparin, polysaccharides, culture media, and more
- Skip RNA and DNA extraction by using crude samples lysates from plants, seeds, cells, bacteria, or FFPE samples
- 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

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

SsoAdvanced Universal Supermixes

| | | SsoAdvanced Universal Inhibitor- Tolerant SYBR® Green Supermix | Quanta Bioscience PerfeCTa SYBR® Green FastMix | Clontech Terra qPCR Direct SYBR® Premix |
|-----------------|------------------------------|---|--|---|
| Sample | Assay | Challenging Target A | Assay (hsGAPDH [226 | bp]; 5 ng hgDNA) |
| Bile salts | Cholic acid | 0.5 μg/μl | 0.25 μg/μl | 0.5 μg/μΙ |
| | Deoxycholic acid | 0.25 μg/μl | 0.125 μg/μl | 0.25 μg/μΙ |
| Polysaccharides | Polygalacturonic acid | 0.625 μg/μl | 0.1563 μg/μl | 0.3125 μg/μl |
| Lipids | Cholesterol hydrochloride | 0.125 μg/μΙ | 0.0625 μg/μl | 0.01563 μg/μl |
| Algae | Alginic acid | 0.25 μg/μΙ | 0.125 μg/μΙ | 0.25 μg/μΙ |
| Clay | Montmorillonite | 20 ng/μl | 20 ng/μl | 20 ng/μl |
| Blood | Heparin | 5 ng/μl | 0 ng/μl | 2.5 ng/µl |
| | Hematin | 1.5625 µM | 0.7813 μM | 0.7813 μM |
| | EDTA | 0.7813 mM | 0.7813 mM | 0.3907 mM |
| | Serum | 0.63% | _ | _ |
| Soil | Humic acid | 0.625 ng/µl | 0.1563 ng/µl | 0.3125 ng/µl |
| Textile | Indigo | 5 mM | 5 mM | 2.5 mM |
| Wine | Tannic acid | 0.6250 ng/µl | 0.3125 ng/µl | 0.3125 ng/µl |
| Plants | Cellulose | 2% | 1% | 1% |
| | Pectin (for fiber control) | 0.125 μg/μΙ | 0.125 μg/μΙ | 0.25 μg/μl |
| Hair, tissues | Melanin | 20 ng/μl | 10 ng/μl | 10 ng/µl |
| Bones, teeth | CaCl ₂ | 1.25 mM | 0.3125 mM | 0.6250 mM |

| | | SsoAdvanced Universal Inhibitor- Tolerant SYBR® Green Supermix | Quanta Bioscience PerfeCTa SYBR® Green FastMix | Clontech Terra qPCR Direct SYBR® Premix |
|---------------|------------------|---|--|---|
| Sample | Assay | Challenging Target A | Assay (hsGAPDH [226 I | op]; 5 ng hgDNA) |
| Cell lysates | MEM+FBS | 5% | 5% | 5% |
| | PBS | 10% | 10% | 5% |
| | Trypsin | 0.000094% | 0.000094% | 0.00% |
| Sample | NaAc | 25 mM | 25 mM | 12.5 mM |
| preparation | NaCl | 12.5 mM | 6.25 mM | 6.25 mM |
| | EtOH | 2.50% | 1.25% | 1.25% |
| | Isopropanol | 2.50% | 1.25% | 1.25% |
| | TRIzol | 0.30% | 0.30% | 0.15% |
| | InstaGene Matrix | 0.30% | 0.15% | 0.02% |
| Miscellaneous | Green tea | 0.50% | - | _ |
| | Chocolate | 0.13% | _ | _ |
| | Dust | 0.18 μg/μl | _ | _ |
| | SDS | 0.003% | 0.003% | 0.006% |
| | DMSO | 3% | 1.50% | 1.50% |
| | DTT | 2 mM | 2 mM | No amplification |
| Body fluids | Spermidine | 1.25 mM | 1.25 mM | 1.25 mM |
| | Urea | 80 mM | 80 mM | 80 mM |

PCR inhibitor tolerance testing with SsoAdvanced Universal Inhibitor-Tolerant SYBR® Green Supermix against Quanta Bioscience PerfeCTa SYBR® Green FastMix and Clontech 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.

4 PCR AND REAL-TIME PCR REAGENTS iTaq Universal Supermixes

iTaq Universal Supermixes

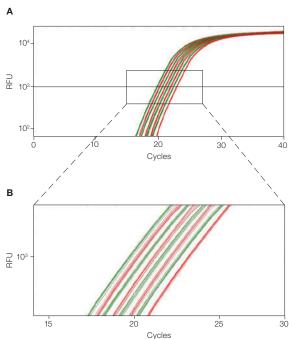
iTaq Universal Supermixes are ready-to-use, 2x reaction master mixes for SYBR® Green– or probe-based qPCR. These supermixes contain an advanced buffer formulation for optimal qPCR performance.

- 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

SYBR 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 System using iTaq Universal SYBR® Green Supermix, which produced 1.33-fold discrimination. RFU, relative fluorescence units.

iTaq Universal Supermixes

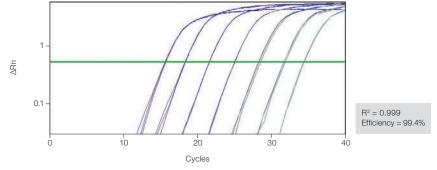
iTaq Universal Supermixes



iTaq Universal Probes Supermix

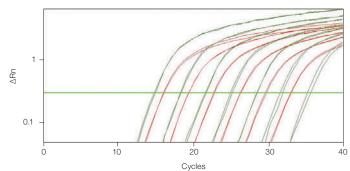
- Carry out high-performance singleplex and duplex reactions robust performance in singleplex or duplex real-time PCR reactions enables increased data precision and saves samples, reagents, and time
- Achieve superior results under various conditions broad range of cycling conditions, primer concentrations, 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

Maximum Dynamic Range for Complete Gene Expression Detection



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.

Advantageous Sensitivity Compared to TaqMan Master Mix



Comparison of dynamic range using iTaq Universal Probes Supermix vs. TaqMan Fast Universal PCR Master Mix (Thermo Fisher Scientific). 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. ∆Rn, baseline-corrected normalized reporter.

PCR AND REAL-TIME PCR REAGENTS Reliance One-Step Multiplex RT-qPCR Supermix



Probe Reliance One-Step Multiplex RT-qPCR Supermix

Reliance One-Step Multiplex Supermix is a ready-to-use, 4x reaction mix optimized for sensitive amplification of up to five targets in a single reaction. Expertly formulated with an optimal enzyme blend, including Reliance Reverse Transcriptase, Sso7d DNA polymerase, and an advanced reaction buffer, this robust supermix delivers reproducible RT-qPCR performance in a multiplex fashion even with challenging templates, difficult targets, or in the presence of PCR inhibitors. With its exceptional room temperature stability for assembled reactions, it can be easily integrated into an automated workflow. Reliance One-Step Multiplex Supermix is an ideal solution for multiplexed RT-qPCR, especially for pathogen/viral detection and high-throughput screening applications.

Key features and benefits of this robust supermix include:

- Sensitive, reproducible multiplexing of up to 5 targets gets the most out of your samples while reducing costs
- Room temperature stability of assembled reaction up to 24 hours enables use in an automated workflow to maximize throughput
- Convenient 1-tube supermix minimizes reaction setup time and handling errors
- 4x concentrated formulation increases detection sensitivity by maximizing input volume
- **Tolerant to common PCR inhibitors** for use with lysates or samples of varying quality



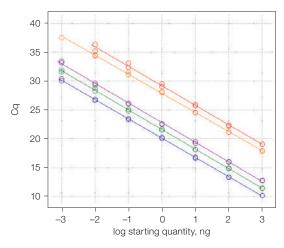


Reliance One-Step Multiplex Supermix allows researchers to:

- Amplify multiple targets with maximum efficiency
- Accelerate data throughput while decreasing costs and reaction time
- Achieve consistent and reproducible data, run after run
- Streamline automated workflows by bypassing the need for plate refrigeration

Reliance One-Step Multiplex RT-qPCR Supermix

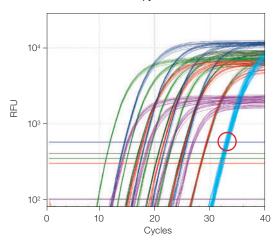
Exceptional Performance of Five-Target Multiplex



| Reporter Dye | Assay | Efficiency, % | R ² | Slope |
|-------------------------------|-------|---------------|----------------|--------|
| - FAM | ACTB | 99.0 | 1.000 | -3.346 |
| - HEX | NGFRP | 100.5 | 0.999 | -3.311 |
| Texas Red | TBP | 97.6 | 0.997 | -3.382 |
| – Cy5 | EF1a | 96.6 | 0.999 | -3.406 |
| - Cy5.5 | GAPDH | 96.9 | 1.000 | -3.397 |

Fig. 1. The linear dynamic range in 5-plex one-step RT-qPCR reactions. Five targets across 7 orders of magnitude (1 pg-1 µg input RNA) were amplified using Reliance One-Step Multiplex Supermix on a CFX96 Touch Real-Time PCR System. The results demonstrated exceptional performance with superior efficiency and linearity over a wide dynamic range down to 1 pg input RNA. Cq, quantification cycle.

Reliable and Sensitive Ten-Copy Detection



| Reporter Dye | Assay | Template | Input Amount(s) |
|-------------------------------|---------|---------------|---|
| - FAM | Control | Synthetic RNA | 10 copies/reaction |
| - FAM | Control | Synthetic RNA | |
| - HEX | NGFRP | HeLa RNA | E 10.5 ()) |
| Texas Red | HSPA4 | HeLa RNA | Four 13.5-fold serial dilutions from 1 µg |
| – Cy5 | EF1a | HeLa RNA | ullutions from 1 µg |
| – Cy5.5 | GAPDH | HeLa RNA | |

Fig. 2. Detection of ten copies of synthetic RNA quantified by one-step reverse transcription Droplet Digital PCR (RT-ddPCR). Fifty-three replicates of ten copies of synthetic RNA (red circle) along with 5-plex reactions consisting of one target from synthetic RNA and four targets from HeLa RNA across the above serial dilutions were amplified using Reliance One-Step Multiplex Supermix. The results demonstrated reliable and sensitive detection of RNA targets down to 10 copies/reaction with tight Cq values between the replicates. RFU, relative fluorescence units.



PCR AND REAL-TIME PCR REAGENTS iTag Universal One-Step Kits

iTag Universal One-Step Kits

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

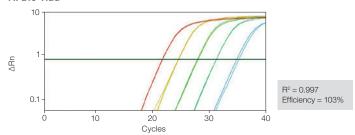
- Achieve superior gene expression results under various cycling conditions — our robust formulation delivers consistent performance in standard or fast cycling conditions, primer concentrations, sample and target types, and temperature ranges, allowing cost and time savings
- Improve PCR efficiency 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

SYBR iTaq Universal SYBR® Green One-Step Kit

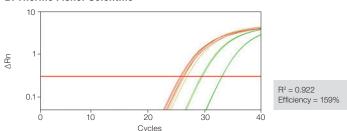
- Benefit from enhanced efficiency, specificity, and sensitivity our patented* inhibitor reducer prevents the interference of the RT enzyme with the DNA polymerase during the replication of cDNA
- Obtain better results with qPCR assays simplify your workflow and experimental design using our wet-lab 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. Thermo Fisher Scientific



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 (Thermo Fisher Scientific). A, iTaq Universal SYBR® Green One-Step Kit; **B**, *Power* SYBR® Green RNA-to-C_τ 1-Step Kit (Thermo Fisher Scientific). Note the delayed threshold cycle (C_τ) values, loss of dynamic range, and compression of input 500 ng total RNA using the *Power* SYBR® Green RNA-to-C_τ 1-Step Kit. This demonstrates RT-qPCR inhibition with higher input RNA and lack of low-level target expression detection. ΔRn, baseline-corrected normalized reporter.

^{*} U. S. patent 8,338,094

iTag Universal One-Step Kits

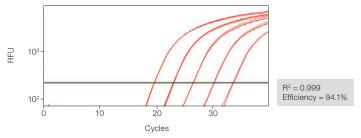


iTag Universal Probes One-Step Kit

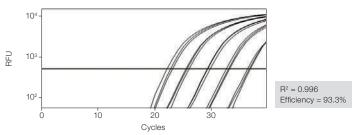
- Obtain superior results with singleplex and multiplex reactions advanced formulation enables the simultaneous amplification of up to 3 targets, resulting in higher data precision with fewer pipetting steps and reduced sample usage
- High-throughput real-time PCR screening and validation simplified 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



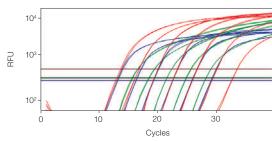
B. Thermo Fisher Scientific



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 System. A, iTaq Universal Probes One-Step Kit; B, TaqMan RNA-to-C_T 1-Step Kit (Thermo Fisher Scientific) produced delayed Cg values with less than optimal data precision for the technical replicates. RFU, relative fluorescence units.

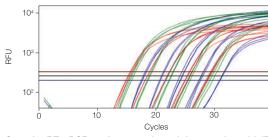
Superior RT-qPCR Performance in a Triplex Reaction

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. Thermo Fisher Scientific



| 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-100 fg RNA was performed on a CFX96 Real-Time PCR 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, TagMan RNA-to-C, 1-Step Kit from Thermo Fisher Scientific 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.

4 PCR AND REAL-TIME PCR REAGENTS iQ Supermixes

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

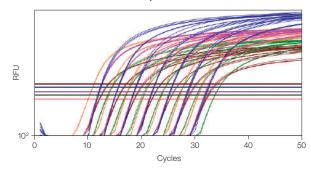
- Robust supermix formulated for sensitive and efficient multiplex gPCR
- Reliable quantification of up to 4 targets (expression levels can vary up to 10⁶-fold between target genes) or up to 5 targets
- Linearity over 6 orders of magnitude of input cDNA and 4 orders of magnitude of input genomic DNA
- Suitable for a wide variety of applications, including gene expression analysis,
 SNP genotyping, SNP analysis, GMO detection, and viral load detection

SYBR

iQSYBR® 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

Accurate and Precise 5-Plex qPCR Results



| Dye | Efficiency, % | Standard Curve, R ² | |
|-----------|---------------|--------------------------------|--|
| FAM | 97.8 | 1.000 | |
| HEX | 98.9 | 1.000 | |
| Texas Red | 99.7 | 1.000 | |
| Cy5 | 98.1 | 1.000 | |
| Quasar | 99.8 | 1.000 | |

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.



78 79

Temperature, °C

Precision Melt Supermix

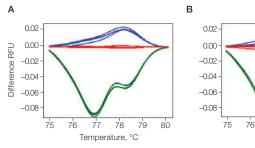
Precision Melt Supermix is a superior high resolution melt (HRM) supermix that delivers optimal discrimination for numerous applications.

- 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 methylamine 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 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

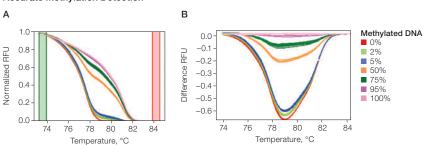
- Genotyping
- Methylation studies
- Mutation detection
- Next-generation sequencing screening
- Species identification

Exceptional Stability



Exceptional stability enables high-throughput genotyping analysis with Precision Melt Supermix. Specific amplification and accurate discrimination of a class IV SNP (84 bp amplicon) from mouse gDNA was performed on a CFX384 Real-Time PCR System either 0 hr (A) or 48 hr (B) after reaction setup. Wild type (I), heterozygote (I), and homozygous mutant (I) 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



Accurate methylation detection with Precision Melt Supermix. A, normalized melt curve; B, difference curve. Mixtures of methylated and unmethylated human gDNA of varying ratios were analyzed using HRM analysis on a CFX384 Real-Time PCR 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.



PCR AND REAL-TIME PCR REAGENTS Precision Blue Real-Time PCR Dye

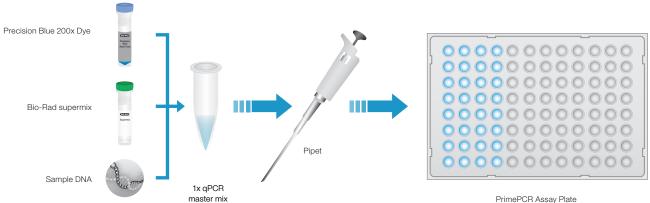
Precision Blue Real-Time PCR Dye

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:

- All PrimePCR Assays and Arrays
- Bio-Rad universal real-time supermixes and one-step kits
- Fluorogenic probes and SYBR® Green chemistries



Pipetting a reaction mix into a PrimePCR Assay Plate.

High-Fidelity and Standard PCR Reagents

Bio-Rad offers reagents for both high-fidelity and standard PCR applications. Use the selection guide to choose an appropriate DNA polymerase for your application.

High-Fidelity and Standard PCR Reagents Selection Guide

| Features | iProof High-Fidelity DNA Polymerase | iTaq DNA Polymerase |
|--------------------------------|--|------------------------|
| Sso7d fusion enzyme technology | Yes | No |
| Hot-start enzyme activation | No | Yes, antibody-mediated |
| 5'→ 3' exonuclease | No | Yes |
| 3'→ 5' exonuclease | Yes | No |
| Blunt or 3'-A ends | Blunt | 3'-A |
| Fidelity (vs. Taq) | 52x | - |
| Long amplification | Yes, up to 37 kb | - |
| Fast cycling | Yes | No |

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

iProof High-Fidelity DNA Polymerase

iProof High-Fidelity DNA Polymerase is composed of a unique *Pyrococcus*-like proofreading enzyme, which employs our patented* Sso7d fusion enzyme technology for speed and accuracy. It is an ideal choice for applications that require high fidelity, such as cloning. iProof DNA Polymerase features:

- High fidelity iProof DNA Polymerase is 52-fold more accurate than Taq DNA polymerase
- **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:

- Convenient, ready-to-use 2x master mix
- DNA polymerase kit, including reaction buffers
- PCR kit, including reaction buffers and controls



^{*} U.S. patents 6,627,424; 7,541,170; and 7,560,260.

4 PCR AND REAL-TIME PCR REAGENTS High-Fidelity and Standard PCR Reagents

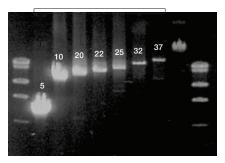
High-Fidelity and Standard PCR Reagents

iProof High-Fidelity DNA Polymerase Performance

Amplification of Long Templates

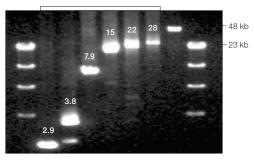
Α

BAC DNA (37 kb)



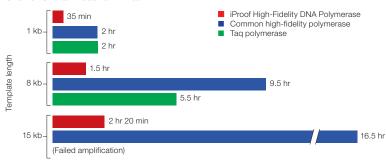
В

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



Total protocol time

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.

Real-Time PCR Reagent Compatibility with Instruments

| | | SYBR® (| Green Supermixes | | | Probes Supe | rmixes | One-Ste | p RT-qPCR Superm | ix and Kits |
|--|--|---|---|-------------------------------|--------------------------|---|---|---|---|--|
| Real-Time PCR Instrument | SsoAdvanced Universal SYBR® Green Supermix | SsoAdvanced Universal Inhibitor- Tolerant SYBR® Green Supermix | iTaq Universal SYBR® Green Supermix | iQ SYBR® Green Supermix | iQ Multiplex Powermix | SsoAdvanced Universal Probes Supermix | iTaq Universal Probes Supermix | Reliance One-Step Multiplex Supermix | iTaq Universal SYBR® Green One-Step Kit | iTaq Universal Probes One-Step Kit |
| Bio-Rad | | - | | | | | | | | |
| CFX96, CFX96 Touch, CFX96 Touch Deep Well, CFX384, CFX384 Touch, CFX Connect | V | V | V | V | V | v | V | V | V | V |
| iQ, iQ5, MyiQ, MyiQ2 | ~ | ✓ | ~ | ~ | ~ | ~ | ~ | ✓ | ~ | ✓ |
| MiniOpticon, DNA Engine Opticon I and II | ✓ | ~ | ✓ | V | ~ | ~ | ~ | ~ | ~ | ✓ |
| Applied Biosystems | | | | | | | | | | |
| StepOne/StepOnePlus | ~ | ~ | ~ | • | • | ~ | ✓ | ~ | ~ | ~ |
| 7500, ViiA 7 | ~ | ~ | ✓ | _ | _ | ~ | ~ | ~ | ✓ | ~ |
| 7000, 7300, 7700, 7900HT, QuantStudio Systems | ✓ | ~ | ✓ | _ | _ | ~ | ~ | ✓ | ~ | V |
| Stratagene | | | | | | | | | | |
| Mx3000P, 3005P, 4000 | ~ | ~ | ✓ | ~ | ✓ | ~ | ~ | ~ | ✓ | ~ |
| Eppendorf | | | | | | | | | | |
| Mastercycler ep realplex 2, 4 | ✓ | ✓ | ~ | V | ~ | ~ | ~ | ~ | ~ | ~ |
| QIAGEN/Corbett | | | | | | | | | | |
| Rotor-Gene 3000, 6000, Q | ✓ | ~ | ✓ | V | ✓ | ~ | V | ~ | V | ✓ |
| Roche | | | | | | | | | | |
| LightCycler 480, 96 | ~ | ~ | ~ | ~ | ✓ | ~ | ~ | ✓ | ~ | ✓ |
| LightCycler 1.0, 1.5, 2.0 | A | A | A | A | A | A | A | A | A | A |
| BioFire | | | | | | | | | | |
| LightScanner HR-1 | ~ | ~ | ✓ | ~ | ✓ | ~ | ~ | ✓ | ~ | ✓ |
| LightScanner 32 | A | A | A | A | A | A | A | A | A | A |

[✓] Recommended for use as is

 ♦ ROX reference setting must be turned off
 ▲ BSA must be added according to instrument specifications
 — Not compatible

4 PCR AND REAL-TIME PCR REAGENTS Ordering Information

Ordering Information

| Cotolog # | December |
|-----------|-------------|
| Catalog # | Description |

SsoAdvanced Universal Supermixes

1,000 x 20 µl reactions

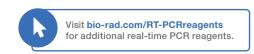
| 000, 10.0 | nood om one our our our |
|-----------|---|
| 1725270 | SsoAdvanced Universal SYBR Green Supermix, 2 ml (2 x 1 ml vials), 200 x 20 µl reactions |
| 1725271 | SsoAdvanced Universal SYBR Green Supermix, 5 ml (5 x 1 ml vials), 500 x 20 µl reactions |
| 1725272 | SsoAdvanced Universal SYBR Green Supermix, 10 ml (10 x 1 ml vials), |
| | 1,000 x 20 µl reactions |
| 1725274 | SsoAdvanced Universal SYBR Green Supermix, 25 ml (5 x 5 ml vials), 2,500 x 20 µl reactions |
| 1725275 | SsoAdvanced Universal SYBR Green Supermix, 50 ml (10 x 5 ml vials), |
| | 5,000 x 20 µl reactions |
| 1725280 | SsoAdvanced Universal Probes Supermix, 2 ml (2 x 1 ml vials), 200 x 20 µl reactions |
| 1725281 | SsoAdvanced Universal Probes Supermix, 5 ml (5 x 1 ml vials), 500 x 20 µl reactions |
| 1725282 | SsoAdvanced Universal Probes Supermix, 10 ml (10 x 1 ml vials), 1,000 x 20 µl reactions |
| 1725284 | SsoAdvanced Universal Probes Supermix, 25 ml (5 x 5 ml vials), 2,500 x 20 µl reactions |
| 1725285 | SsoAdvanced Universal Probes Supermix, 50 ml (10 x 5 ml vials), 5,000 x 20 µl reactions |
| 1725016 | SsoAdvanced Universal Inhibitor-Tolerant SYBR Green Supermix, 2 ml (2 x 1 ml vials), |
| | 200 x 20 µl reactions |
| 1725017 | $\textbf{SsoAdvanced Universal Inhibitor-Tolerant SYBR Green Supermix}, 5 \ \text{ml (1} \times 5 \ \text{ml vial)},$ |
| | 500 x 20 ul reactions |

1725018 SsoAdvanced Universal Inhibitor-Tolerant SYBR Green Supermix, 10 ml (2 x 5 ml vials),

Catalog # Description

iTaq Universal Supermixes

| 1725120 | iTaq Universal SYBR Green Supermix, 2 ml (2 x 1 ml vials), 200 x 20 μl reactions |
|------------|---|
| 1725121 | iTaq Universal SYBR Green Supermix, 5 ml (5 x 1 ml vials), 500 x 20 μl reactions |
| 1725122 | iTaq Universal SYBR Green Supermix, 10 ml (10 x 1 ml vials), 1,000 x 20 μl reactions |
| 1725124 | iTaq Universal SYBR Green Supermix, 25 ml (5 x 5 ml vials), 2,500 x 20 μl reactions |
| 1725125 | iTaq Universal SYBR Green Supermix, 50 ml (10 x 5 ml vials), 5,000 x 20 μl reactions |
| 1725130 | iTaq Universal Probes Supermix, 2 ml (2 x 1 ml vials), 200 x 20 μl reactions |
| 1725131 | iTaq Universal Probes Supermix, 5 ml (5 x 1 ml vials), 500 x 20 μl reactions |
| 1725132 | iTaq Universal Probes Supermix, 10 ml (10 x 1 ml vials), 1,000 x 20 μl reactions |
| 1725134 | iTaq Universal Probes Supermix, 25 ml (5 x 5 ml vials), 2,500 x 20 μl reactions |
| 1725135 | iTaq Universal Probes Supermix, 50 ml (10 x 5 ml vials), 5,000 x 20 μl reactions |
| Reliance 0 | One-Step Multiplex RT-qPCR Supermixes |
| 12010176 | Reliance One-Step Multiplex Supermix, 1 ml (1 x 1 ml vial), 200 x 20 µl reactions |
| 12010220 | Reliance One-Step Multiplex Supermix, 5 ml (5 x 1 ml vials), 1,000 x 20 µl reactions |
| 12010221 | Reliance One-Step Multiplex Supermix, 10 ml (2 x 5 ml vials), 2,000 x 20 µl reactions |
| iTaq Unive | ersal One-Step RT-qPCR Kits |
| 1725150 | iTaq Universal SYBR Green One-Step Kit, 1 ml (1 x 1 ml vial), 100 x 20 μl reactions |
| 1725151 | iTaq Universal SYBR Green One-Step Kit, 5 ml (5 x 1 ml vials), 500 x 20 μ l reactions |
| 1725140 | iTaq Universal Probes One-Step Kit, 1 ml (1 x 1 ml vial), 100 x 20 μ l reactions |
| 1725141 | iTaq Universal Probes One-Step Kit, 5 ml (5 x 1 ml vials), 500 x 20 μ l reactions |
| | |

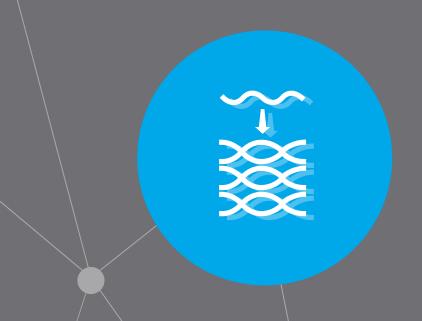


PCR AND REAL-TIME PCR REAGENTS Ordering Information

| Catalog # | Description | Catalog # | Description |
|--|---|--|---|
| iQ Superr | nixes | Standard | PCR Reagents |
| 1725848 | iQ Multiplex Powermix, 1.25 ml (1 x 1.25 ml vial), 50 x 50 μl reactions | 1725310 | iProof HF Master Mix, 0.04 U/μl, 100 x 50 μl reactions |
| 1725849 | iQ Multiplex Powermix, 5 ml (4 x 1.25 ml vials), 200 x 50 µl reactions | 1725320 | iProof GC Master Mix, 0.04 U/μl, 100 x 50 μl reactions |
| 1708880 1708882 1708884 1708885 1708886 1708887 1708860 1708862 | iQ SYBR Green Supermix, 2.5ml ($2 \times 1.25 \text{ml}$ vials), $100 \times 50 \mu \text{l}$ reactions iQ SYBR Green Supermix, 12.5ml ($10 \times 1.25 \text{ml}$ vials), $500 \times 50 \mu \text{l}$ reactions iQ SYBR Green Supermix, 25ml ($20 \times 1.25 \text{ml}$ vials), $1,000 \times 50 \mu \text{l}$ reactions iQ SYBR Green Supermix, 50ml (50ml bottle), $2,000 \times 50 \mu \text{l}$ reactions iQ SYBR Green Supermix, 25ml ($5 \times 5 \text{ml}$ vials), $1,000 \times 50 \mu \text{l}$ reactions iQ SYBR Green Supermix, 50ml ($10 \times 5 \text{ml}$ vials), $2,000 \times 50 \mu \text{l}$ reactions iQ Supermix, 2.5ml ($2 \times 1.25 \text{ml}$ vials), $100 \times 50 \mu \text{l}$ reactions iQ Supermix, 12.5ml ($10 \times 1.25 \text{ml}$ vials), $100 \times 50 \mu \text{l}$ reactions | 1725301 1725302 1725330 1725331 1708870 1708875 1725858 1708874 Additiona 1725110 1725112 1725555 | iProof High-Fidelity DNA Polymerase, includes 5x reaction buffers, MgCl ₂ , and DMSO, 100 U, 2 U/µl iProof High-Fidelity DNA Polymerase, includes 5x reaction buffers, MgCl ₂ , and DMSO, 500 U, 2 U/µl iProof High-Fidelity PCR Kit, includes 5x reaction buffers and DNA control, 50 U, 2 U/µl iProof High-Fidelity PCR Kit, includes 5x reaction buffers and DNA control, 200 U, 2 U/µl iTaq DNA Polymerase, 250 U, 5 U/µl iTaq DNA Polymerase, 5,000 U, 5 U/µl ROX Passive Reference Dye, 0.5 ml dNTP Solution Mix, 200 µl IReal-Time PCR Reagents Precision Melt Supermix, 2 ml (2 x 1 ml vials), 200 x 20 µl reactions Precision Blue Real-Time PCR Dye, 55 µl (1 x 1 ml vial), 500 x 20 µl reactions |
| | | 1720000 | Frecision blue near-time Fon Dye, 33 pt (1 x 1 mt wal), 300 x 20 pt reactions |



5 PREAMPLIFICATION



Preamplification is a powerful tool to enable highly sensitive detection of a large number of targets from a limited sample.

- Proprietary buffers and Sso7d fusion technology generate unbiased preamplification reactions
- Optimized for PrimePCR PreAmp Assays
- Unparalleled sensitivity

Preamplification Selection Guide

Preamplification is a valuable tool that enables researchers to enrich specific gene targets of interest from a limited sample. Selecting a preamplification kit that provides unbiased preamplification is key to successful gene expression analysis.

| Product | Description | Key Benefits |
|--|---|---|
| iScript Explore One-Step RT and PreAmp Kit | cDNA synthesis and preamplification performed in a single step RNA used as input sample Reverse transcription carried out using gene-specific priming | Unbiased preamplification Protocol minimizes hands-on time Compatible with PrimePCR PreAmp Control Kit integrates an effective gDNA digestion step |
| SsoAdvanced PreAmp Supermix | cDNA synthesis and preamplification performed in separate steps cDNA synthesis carried out using any of the iScript family of reverse transcription reagents cDNA used as input sample for preamplification reaction Reverse transcription carried out using gene-specific priming | Unbiased preamplification Flexibility with cDNA synthesis method of choice cDNA can be archived for future studies of different genes |

Ideal Sample Types for Preamplification

- FFPE samples
- Single cells
- LCM samples
- Small biopsies
- Plant samples
- Sorted cells
- Rare samples
- Stem cells

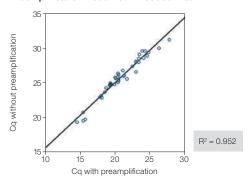
5 PREAMPLIFICATION iScript Explore One-Step RT and PreAmp Kit

iScript Explore One-Step RT and PreAmp Kit

iScript Explore One-Step RT and PreAmp Kit features:

- 1-step reverse transcription and preamplification the first kit that eliminates protocol steps by generating preamplified cDNA from RNA in a single step
- Unbiased preamplification can reliably profile panels of targets
- **High sensitivity** enables reliable detection of genes expressed at low levels
- Preamplification control preamplification efficacy can be easily assessed when used with PrimePCR Long Noncoding RNA (IncRNA) Arrays or custom PrimePCR Plates
- Effective genomic DNA removal provides confidence that results are only for the transcript of interest

Preamplification Does Not Introduce Bias



High correlation between preamplified and non-preamplified samples. Potential for bias was evaluated using ERCC RNA transcripts of a known copy number. ERCC concentrations from single copy to 16,000 copies were used to assess bias across a wide range of transcript concentrations. RNA samples were reverse transcribed using iScript Advanced cDNA Synthesis Kit for RT-qPCR or reverse transcribed and preamplified using iScript Explore One-Step RT and PreAmp Kit. The R² value of 0.952 indicates a high correlation between samples with and without preamplification, demonstrating that preamplification does not introduce bias. Cq, quantification cycle.





SsoAdvanced PreAmp Supermix

SsoAdvanced PreAmp Supermix provides unbiased, target-specific preamplification from limited nucleic acids using up to 100 gPCR or end-point assays and enabling more downstream reactions for screening of respective targets.

Features of SsoAdvanced PreAmp Supermix

- Unbiased preamplification for accurate expression analysis from limited sample
- 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 TagMan and custom assays
- Superior sensitivity and efficiency
- Economically priced

Ordering Information

| Catalog # | Description |
|--------------|---|
| iScript Expl | lore One-Step RT and PreAmp Kits |
| 12004856 | iScript Explore One-Step RT and PreAmp Kit, 50 x 50 µl reactions |
| 17002826 | iScript Explore One-Step RT and PreAmp Kit, 250 x 50 µl reactions |
| SsoAdvanc | ed PreAmp Supermix |
| 1725160 | SsoAdvanced PreAmp Supermix, 1.25 ml (1 x 1.25 ml vial), 50 x 50 µl reactions |

This guide provides a comparison of PreAmp products.

| Property | SsoAdvanced PreAmp Supermix | TaqMan PreAmp Master Mix (Thermo Fisher Scientific) | PerfeCTa PreAmp 5x SuperMix (Quanta BioSciences) |
|---|--------------------------------|--|--|
| Bias quantification score | 90% ≤ 0.75 | 90% ≤ 1.50 | 90% ≤ 1.50 |
| Sensitive detection of low-level target genes | ••• | •• | • |
| High efficiency, even for difficult amplicons | ••• | •• | •• |
| Validated assays | PrimePCR PreAmp, TaqMan | TaqMan | Unknown |
| SYBR® Green Assays | ✓ | | ✓ |
| Probe assays | ✓ | ✓ | ✓ |
| Number of reactions | 50 x 50 μl | 40 x 50 µl | 40 x 50 μl |

^{••} Good • • • Excellent

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.



6 PCR PLASTIC CONSUMABLES

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

| | | Tubes | |
|---|---------------------------|---------------------|--------------------|
| | Individual High-Profile | Strips High-Profile | Strips Low-Profile |
| Catalog # | TBI0201, TFI0201, TWI0201 | TBCxxxx, TBSxxxx | TLSxxxx |
| Thermal Cycler | | | |
| Bio-Rad C1000, C1000 Touch, S1000 | ✓ | A | √ |
| Bio-Rad DNA Engine, DNA Engine Tetrad, DNA Engine Tetrad 2, DNA Engine Dyad, Dyad Disciple, PTC-100 | V | A | ~ |
| Bio-Rad T100, MyCycler | ✓ | ✓ | |
| Bio-Rad iCycler | ✓ | ✓ | |
| Bio-Rad MJ Mini | ✓ | A | <i>V</i> |
| Applied Biosystems 0.2 ml Tube Cyclers (2720, 9700, Veriti) | ✓ | v | |
| Applied Biosystems 0.1 ml Tube Cyclers (9800 Fast, Veriti Fast) | | | ✓ |
| Eppendorf Mastercycler Series | ✓ | A | ✓ |
| Real-Time PCR Instrument | | | |
| CFX Opus 96, CFX Opus 384 | | | ✓ |
| Bio-Rad CFX96, CFX96 Touch, CFX96 Touch Deep Well, CFX Connect | | | √ |
| Bio-Rad iCycler iQ, iQ5, MyiQ, MyiQ2 | | ✓ | |
| Bio-Rad Chromo4 | | A | √ |
| Bio-Rad DNA Engine Opticon, Opticon 2 | | | ✓ |
| Bio-Rad MiniOpticon* | | | ✓ |
| Applied Biosystems Standard Systems (7300, 7500, 7900HT) | | <i>v</i> | |
| Applied Biosystems Fast Systems (7500 Fast, 7900HT Fast, StepOne, StepOnePlus) | | | ✓ |
| Eppendorf Mastercycler ep realplex | | A | v |
| Stratagene (Agilent) Mx Series | | √ | |
| QIAGEN/Corbett Rotor-Gene | ✓ | | |

^{*} The MiniOpticon Real-Time PCR 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.

6 PCR PLASTIC CONSUMABLES Instrument Compatibility

| | | | 384-Well Plates | | | | | | |
|--|--------------------------------------|--|--|-------------------|---|--|------------------------------------|------------------------|-------------------|
| | Hard-Shell Skirted Low-Profile | Hard-Shell Semi-Skirted High-Profile | Hard-Shell Semi-Skirted Low-Profile | Hard-Shell 480 | Multiplate Unskirted High-Profile | Multiplate Unskirted Low-Profile | iQ Semi-Skirted High-Profile | Hard-Shell Standard | Hard-Shell 480 |
| Catalog # | HSP9xxx | HSS9xxx | HSL9xxx | HSR99xx | MLPxxxx | MLLxxxx | 2239441 | HSP3xxx | HSR48xx |
| Thermal Cycler | | | | | | | | | |
| Bio-Rad C1000, C1000 Touch, S1000 | ~ | A | A | | A | A | A | ~ | A |
| Bio-Rad T100 | | V | | | A | | A | | |
| Bio-Rad DNA Engine, DNA Engine Tetrad, DNA Engine Tetrad 2, DNA Engine Dyad | ~ | A | | | A | A | A | V | A . |
| Bio-Rad iCycler, MyCycler | | Except MyCycler | | | V | | A | | |
| Bio-Rad MJ Mini | | | | | A | ✓ | | | |
| Applied Biosystems 0.2 ml Tube Cyclers (2720, 9700, Veriti) | | ✓ | | | A | | A | | |
| Applied Biosystems 0.1 ml Tube Cyclers (9800 Fast, Veriti Fast) | | | ~ | | | ~ | | | |
| Applied Biosystems 384-Well Cyclers (9700, Veriti) | | | | | | | | V | A |
| Eppendorf Mastercycler Series | V | A | A | | A | A | A | ~ | A |
| Real-Time PCR Instrument | | | | | | | | | |
| Bio-Rad CFX Opus 96, CFX Opus 384 | ~ | | A | | | | | ~ | |
| Bio-Rad CFX96, CFX96 Touch, CFX Connect, CFX384,* CFX384 Touch* | ~ | | A | | | A | | V | A |
| Bio-Rad CFX96 Touch Deep Well | V | A | | | A | | | | |
| Bio-Rad iCycler iQ, iQ5, MyiQ, MyiQ2 | | A | | | A | | V | | |
| Bio-Rad DNA Engine Opticon, Opticon 2, MiniOpticon* | Except MiniOpticon | | | | | A | | | |
| Applied Biosystems Standard Systems (7300, 7500, 7900HT, QuantStudio Systems, ViiA 7) | | V | | | Except 7900HT, QuantStudio | | Except 7900HT, QuantStudio | V | • |
| Applied Biosystems Fast Systems (7500 Fast, 7900HT Fast, StepOne, StepOnePlus, QuantStudio Systems, ViiA 7 Fast) | | | Except StepOne, StepOnePlus, 7900HT Fast | | | Except 7900HT Fast | | V | A |
| Eppendorf Mastercycler ep realplex | V | A | A | | A | A | A | | |
| Stratagene (Agilent) Mx Series | | ~ | | | A | | A | | |
| Roche LightCycler 96, 480 | | | | ~ | | | | | V |

Contact Bio-Rad technical support for compatibility with additional instruments.



^{*} CFX384, CFX384 Touch, and MiniOpticon Real-Time PCR 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



ECT2000 Strip Cap Tool

TLS0801, clear TLS0851, white Low-Profile Tube Strips without Caps

TBS0201, 8-tube, clear TBS1201, 12-tube, clear High-Profile Tube Strips without Caps

TCS0803, ultraclear Optical Flat 8-Cap Strips

TCS0801, 8-cap, clear TCS1201, 12-cap, clear Domed Cap Strips

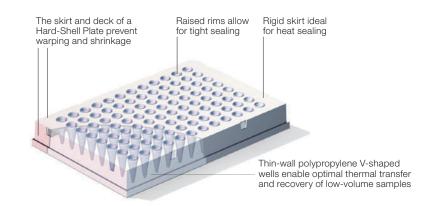
6 PCR PLASTIC CONSUMABLES Hard-Shell PCR Plates

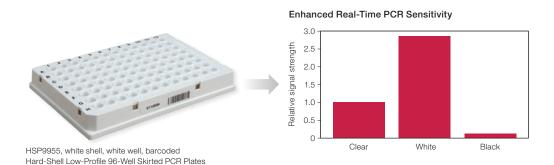
Hard-Shell PCR Plates

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



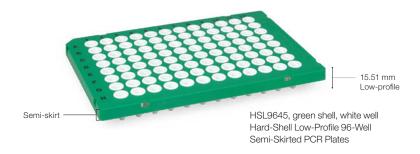




Other colors and barcode option are available.

PCR PLASTIC CONSUMABLES 6 Hard-Shell PCR Plates

Hard-Shell PCR Plates











6 PCR PLASTIC CONSUMABLES PCR Plates

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)



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 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



REPORT 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



MSB1001 Microseal 'B' Adhesive Seals



MSC1001 Microseal 'C' Optical Seals

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



MSA5001 Microseal 'A' Film



MSF1001 Microseal 'F' Foil



Sealing Roller

Ordering Information

Catalog # Description

PCR Plate Sealing

- 1814000 PX1 PCR Plate Sealer, includes heat sealing instrument, 96-well/384-well plate support block,
 - sealing frame, power cord
- 1814030 Optically Clear Heat Seal, 100 seals 1814035 Permanent Clear Heat Seal, 100 seals
- 1814040 Pierceable Foil Heat Seal. 100 seals
- 1814045 Peelable Foil Heat Seal, 100 seals
- MSA5001 Microseal 'A' Film, 50 seals
- MSB1001 Microseal 'B' Adhesive Seals, optically clear, 100 seals
- MSC1001 Microseal 'C' Optical Seals, 100 seals
- MSF1001 Microseal 'F' Foil, 100 seals
- MSR0001 Sealing Roller, for film seals

PCR PLASTIC CONSUMABLES 6 Ordering Information

Ordering Information

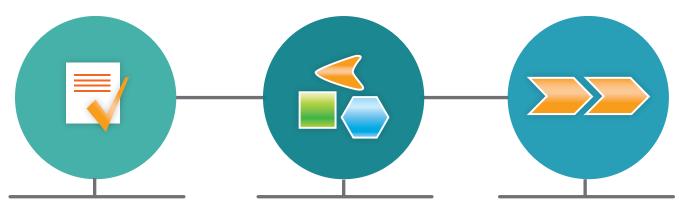
| Catalog # | Description | Description | Clear Well | White Well | Black Well | | | |
|-----------------------|--|---|---------------------------|--------------------|--|--|--|--|
| ndividual | PCR Tubes | Hard-Shell Plates | | | | | | |
| ΓFI0201 | PCR Tubes with Flat Caps (0.2 ml), clear, 1,000 | Hard-Shell Low-Profile 96- | Well Skirted PCR Plates (| 50 plates) | | | | |
| ΓWI0201 | PCR Tubes with Domed Caps (0.2 ml), clear, 1,000 | White shell | HSP9601 | HSP9655 | _ | | | |
| ГВ10201 | PCR Tubes without Caps (0.2 ml), clear, 1,000 | Red shell | HSP9611 | _ | _ | | | |
| ГВІ0501 | PCR Tubes with Flat Caps (0.5 ml), clear, 1,000 (2 bags of 500) | Yellow shell | HSP9621 | _ | _ | | | |
| ΓΒI0502 | PCR Tubes with Flat Caps (0.5 ml), clear, 800 (8 bags of 100) | Blue shell | HSP9631 | HSP9635 | _ | | | |
| PCR Tube | Strips | Green shell | HSP9641 | HSP9645 | _ | | | |
| ΓBS0201 | 8-Tube Strips without Caps, clear, 120 strips (960 PCR tubes) | Black shell | HSP9661 | HSP9665 | HSP9666 | | | |
| ΓBS1201 | 12-Tube Strips without Caps, clear, 100 strips (1,200 PCR tubes) | White shell, barcoded | HSP9901 | HSP9955 | _ | | | |
| ΓBC0802 | 8-Tube Strips and Domed Cap Strips, clear, 20 bags of 12 x 8-tube strips | (Row H and Column 12) | | | | | | |
| | and 12 x 8-cap strips (1,920 PCR tubes and 1,920 caps) | Hard-Shell High-Profile 96 | | lates (25 plates) | | | | |
| ГВС1202 | 12-Tube Strips and Domed Cap Strips, clear, 20 bags of 8 x 12-tube strips | Clear shell | HSS9601 | _ | _ | | | |
| TLS0801 | and 8 x 12-cap strips (1,920 PCR tubes and 1,920 caps) | Green shell | HSS9641 | _ | _ | | | |
| TLS0851 | Low-Profile 8-Tube Strips without Caps, clear, 120 (960 PCR tubes) Low-Profile 8-Tube Strips without Caps, white, 120 (960 PCR tubes) | Black shell | _ | HSS9665 | _ | | | |
| | | Clear shell, barcoded | HSS9901 | _ | _ | | | |
| Cap Strips FCS0801 | | (Row H, Column 1, and Colur Hard-Shell Low-Profile 96- | , | lates (25 plates) | | | | |
| TCS0601 | Domed 8-Cap Strips, for PCR tubes and plates, clear, 120 | Clear shell | HSL9601 | ` ' ' | | | | |
| | Domed 12-Cap Strips, for PCR tubes and plates, clear, 200 | | | HSL9605 | _ | | | |
| rcso8o3 | Optical Flat 8-Cap Strips, for PCR tubes and plates, ultraclear, 120 | Green shell Clear shell, barcoded | HSL9641 HSL9901 | HSL9645 HSL9905 | _ | | | |
| | ools and Racks | (Row H and Column 12) | 11019901 | 11329903 | _ | | | |
| FRC9601 FRC0501 | PCR Tube Rack, ANSI/SBS standard, white, 10 96-Place Racks, with covers, for PCR tubes and unskirted and semi-skirted | Hard-Shell 384-Well Standard PCR Plates (50 plates) | | | | | | |
| 11100301 | microplates, assorted colors, 5 | Clear shell | HSP3801 | HSP3805 | _ | | | |
| ECT1000 | Easy Cap Tool, ensures tight seal for 0.2 ml PCR tubes | Red shell | HSP3811 | _ | _ | | | |
| | or 96-well microplates | Yellow shell | HSP3821 | _ | _ | | | |
| ECT2000 | Strip Cap Tool, for sealing 8- and 12-cap strips on PCR plates or tubes | Blue shell | HSP3831 | _ | _ | | | |
| | | Green shell | HSP3841 | _ | _ | | | |
| PCR Plate | | Black shell | _ | HSP3865 | HSP3866 | | | |
| | 48-Well PCR Plates | Clear shell, barcoded | HSP3901 | HSP3905 | _ | | | |
| MLP4801 | Multiplate High-Profile 48-Well Unskirted PCR Plates, clear, 50 plates | (Row P and Column 24) | | | | | | |
| MLL4801 | Multiplate Low-Profile 48-Well Unskirted PCR Plates, clear, 50 plates | Description | | Number of Plates | Number of Plates and Seals | | | |
| MLL4851 | Multiplate Low-Profile 48-Well Unskirted PCR Plates, white, 50 plates | | | | 100 Plates, 100 | | | |
| • | High-Profile 96-Well Unskirted PCR Plates | | | 25 Plates | Microseal 'C' Seals | | | |
| MLP9601 | Multiplate High-Profile 96-Well Unskirted PCR Plates, clear, 25 plates | Hard-Shell 96-Well 480 PC | | | | | | |
| MLP9651 | Multiplate High-Profile 96-Well Unskirted PCR Plates, white, 25 plates | (with barcode on Row A si | de) | LIGDOGG | LIODOGGELA | | | |
| MLP9631 | Multiplate High-Profile 96-Well Unskirted PCR Plates, blue, 25 plates | Clear shell, white well | | HSR9905 | HSR9905K | | | |
| • | Low-Profile 96-Well Unskirted PCR Plates | Clear shell, clear well | | HSR9901 | HSR9901K | | | |
| MLL9601 | Multiplate Low-Profile 96-Well Unskirted PCR Plates, clear, 25 plates | | | 50 Plates | 100 Plates, 100 Microseal 'C' Seals | | | |
| MLL9651 | Multiplate Low-Profile 96-Well Unskirted PCR Plates, white, 25 plates | Hard-Shell 384-Well 480 PC | CR Plates | 55 i idio5 | occar o ocaro | | | |
| | rofile 96-Well Semi-Skirted PCR Plates | (with barcode on Row A ar | nd Column 24 side) | | | | | |
| 2239441 | iQ High-Profile 96-Well Semi-Skirted PCR Plates, 25 plates | Clear shell, white well | | HSR4805 | HSR4805K | | | |
| | | Clear shell, clear well | | HSR4801 | HSR4801K | | | |



PrimePCR Assays and Panels for real-time PCR are expertly designed and are guaranteed to perform.

- Expertly designed SYBR® Green and probe assays for qPCR, preamplification, and Droplet DigitalPCR
- Wet-lab validated for guaranteed performance; each mRNA assay for the human, mouse, and rat genomes was experimentally tested for optimal efficiency, specificity, sensitivity, and linear dynamic range
- Assays available for expression analysis of messenger RNA and long noncoding RNA targets
- Compliant with the MIQE guidelines

High-Performance Assay Solutions



Guaranteed Performance

- Offers wet-lab validation of every mRNA assay for human, mouse, and rat genomes
- Eliminates time-consuming optimization
- Aids in MIQE compliance

Wide Range of Predesigned Diseaseand Pathway-Specific Panels

- Expertly curated predesigned arrays to include the most biologically relevant gene targets
- Customizable arrays to select targets of interest
- Integrated with CFX Maestro Software for simple setup and analysis

Complete Solution for Real-Time PCR

- Aurum Total RNA Kits
- iScript cDNA Synthesis Kits
- Supermixes for qPCR
- Preamplification reagent kits and PrimePCR PreAmp Assays
- PCR plates and tubes
- Real-time PCR instruments
- CFX Maestro Software
- PrimePCR Analysis Software

Assay Design

Advantages

- Assay specificity confirmed by next-generation sequencing
- Avoided common single nucleotide polymorphisms in target regions
- Designed intronspanning assays whenever possible
- Avoided secondary structures in primer annealing sites
- Maximized fraction of transcript isoforms being detected
- Compatible with standard assay conditions

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 with five fluorophore options.





Custom Assays

User-defined primer and probe sequences can be ordered.



PreAmp Assays

Concentrated primers are available for target-specific preamplification of limited nucleic acid samples.



DNA Templates

Gene-specific synthetic DNA templates are designed to serve as a positive control 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

Thousands of PrimePCR Assays are available to create custom-configured 96- and 384-well PCR plates, which can be ordered with SYBR® Green Assays.



PrimePCR Lookup Tool

Quickly find your gene targets. Visit bio-rad.com/PrimePCR to view our full offering of high-performance PrimePCR Assays.



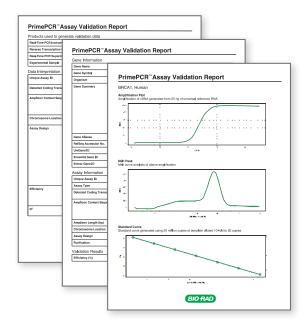
Assay Performance Standards and Validation

Assay Performance Standards

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

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 System.



7 PrimePCR ASSAYS AND PANELS Predesigned mRNA Pathway Panels

Predesigned mRNA 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
- Gain new insights using integrated data analysis tools



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



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.

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
- Eve 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/ PrimePCRpanels for more information.

7 PrimePCR ASSAYS AND PANELS

Predesigned and Custom PrimePCR Long Noncoding RNA (IncRNA) Arrays

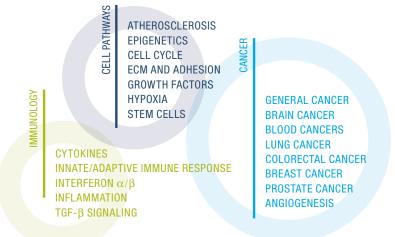
Predesigned and Custom PrimePCR Long Noncoding RNA (IncRNA) Arrays

Simplified Long Noncoding RNA Discovery

Predesigned PrimePCR IncRNA qPCR Arrays facilitate IncRNA discovery by offering panels associated with specific research areas. Each plate was curated to include a combination of frequently cited IncRNAs as well as novel IncRNAs to aid in target discovery.

- Faster preamplification setup with matching PrimePCR IncRNA PreAmp Pools
- Extensively wet-lab validated to ensure stringent performance standards are met by IncRNA assays on predesigned plates
- Great companion for predesigned PrimePCR mRNA Arrays

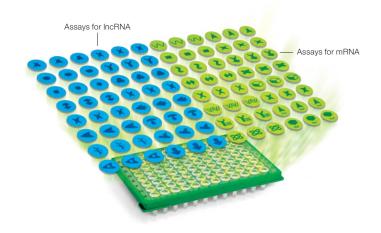
Available IncRNA Arrays



Create Your Own Long Noncoding RNA Array

Custom PrimePCR IncRNA qPCR Arrays simplify IncRNA expression research. Easily expand upon next-generation sequencing and literature data mining projects by using qPCR arrays tailored to your specific research question. Our custom qPCR arrays help move your research forward by providing a well-accepted, highly sensitive, and cost-effective approach to gene expression research. Custom qPCR arrays enable you to go from RNA sample through data analysis in a single day.

- Choose from more than 23,000 IncRNAs to customize array plates with only your targets of interest
- Simultaneously examine both IncRNA and mRNA expression patterns
- Design and include your own PCR primers on any custom plate



PrimePCR Long Noncoding RNA Assays

Assay Formats to Fit Your Needs



IncRNA SYBR® Green Primer Assays



IncRNA Probes Assays compatible with multiplexing. Available reporter dyes: FAM, HEX, Texas Red 615, Cy5, and Cy5.5



 Concentrated PreAmp Assays that can be used for both preamplification and downstream qPCR reactions



DNA assay templates for every human lncRNA assay serve as a positive control

 $\label{localization} \mbox{PrimePCR IncRNA Assays are also compatible with Droplet Digital PCR workflows.}$

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.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | , |
|----|---|----|----|----|----|----|----|----|----|----|--------|--------|---|
| \ | 1 | 9 | 17 | 25 | 33 | 41 | 49 | 57 | 65 | 73 | 81 | 89/Ref | A |
| В | 2 | 10 | 18 | 26 | 34 | 42 | 50 | 58 | 66 | 74 | 82 | PAQ 1 | В |
| С | 3 | 11 | 19 | 27 | 35 | 43 | 51 | 59 | 67 | 75 | 83 | PAQ 2 | С |
| D | 4 | 12 | 20 | 28 | 36 | 44 | 52 | 60 | 68 | 76 | 84 | gDNA | D |
| E | 5 | 13 | 21 | 29 | 37 | 45 | 53 | 61 | 69 | 77 | 85 | PCR | E |
| F | 6 | 14 | 22 | 30 | 38 | 46 | 54 | 62 | 70 | 78 | 86 | RQ 1 | F |
| G | 7 | 15 | 23 | 31 | 39 | 47 | 55 | 63 | 71 | 79 | 87 | RQ2 | G |
| 4 | 8 | 16 | 24 | 32 | 40 | 48 | 56 | 64 | 72 | 80 | 88/Ref | RT | н |
| Ĺ, | 1 | 2 | 3 | _4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |

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.

Preamplification Quality Assay

PAQ 1

PCR

The PrimePCR Preamplification Quality Assay is designed to work with iScript Explore One-Step RT and PreAmp Kit to determine the efficiency of preamplification.

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 Assav

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 Maestro 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 Maestro 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
- View data in the PrimePCR Controls Analysis Tool and Reference Gene Selection Tool in CFX Maestro Software to easily view results of controls and identify ideal reference genes for your experiment

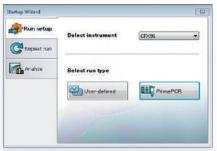
PrimePCR Runs from Start to Finish

Start runs quickly by choosing **PrimePCR** in the Startup Wizard to select the validated PrimePCR run protocol, then click **Start Run**. CFX Maestro 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/PrimePCRsoftware** to download the software. Easily upload Cq values and quickly generate meaningful information from your gene expression experiment.



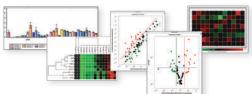


Apply run file



The run file autopopulates the plate layout with gene names, making multiplate data analysis quick and simple.

3 Automated data analysis





Use PrimePCR Analysis Software! bio-rad.com/PrimePCRsoftware

8 PCR RUN AND DATA ANALYSIS

Bio-Rad 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 systems efficiently provide accurate, reliable data
- CFX Maestro Software enables the collection and in-depth 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 Systems

Bio-Rad real-time PCR systems are powerful, flexible, and accurate instruments for both singleplex and multiplex detection of fluorophores. The systems feature thermal gradient functionality and automation capabilities.

- CFX Opus 96 Real-Time PCR System
- CFX Opus 384 Real-Time PCR System
- CFX96 Touch Deep Well Real-Time PCR System
- CFX Connect Real-Time PCR System



Designed for compatibility and integration. CFX Opus Real-Time PCR Systems are automation and integration ready. CFX Maestro Software has an application programming interface that allows for integration with laboratory information management systems and configuration into other custom software solutions.

CFX Maestro Software

CFX Maestro Software for CFX real-time PCR systems is easy-to-use. This flexible and powerful software performs data collection, data analysis, and graphing of real-time PCR data. You can view and analyze the most relevant data in one screen.

With CFX Maestro Software you can:

- Perform automatic statistical analysis in seconds with just a few mouse clicks you can perform t-tests or analyze your data with 1-way analysis of variance (ANOVA)
- **Extract more meaningful information from your run** analyze data using bar chart, box and whisker plot, dot plot, clustergram, scatter plot, or volcano plot
- Create and export publication-ready graphics annotate graphs with P values, text, and arrows to call out specific data. Change colors, fonts, and legends. Export graphs at any size or resolution for presentations, posters, or for publication
- Easily integrate PrimePCR Assays use PrimePCR Primers and Plates to save time on primer design with predesigned and validated primers. Post-run, use the PrimePCR Controls Analysis Tool to ensure run quality from integrated controls
- Work anywhere, on a PC or Mac with both PC and Mac versions of CFX
 Maestro Software, you can analyze your data on your own computer, anytime,
 without the need for an Internet connection (Mac version is for data analysis only
 and does not provide instrument control)



For more information about CFX systems, download bulletins 5990, 6105, and 7279.



For more information about CFX Maestro Software, download bulletin 6900.

Reference

Bustin SA et al. (2009). The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. Clin Chem 55, 611-622.

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

BIO-RAD, DROPLET DIGITAL PCR, HARD-SHELL, and MICROSEAL are trademarks of Bio-Rad Laboratories, Inc. in certain jurisdictions. All trademarks used herein are the property of their respective owner.

EvaGreen is a trademark of Biotium, Inc. Bio-Rad Laboratories, Inc. is licensed by Biotium, Inc. to sell reagents containing EvaGreen Dye for use in real-time PCR, for research purposes only.

SYBR is a trademark of Thermo Fisher Scientific Inc. Bio-Rad Laboratories, Inc. is licensed by Thermo Fisher Scientific Inc. to sell reagents containing SYBR Green I for use in real-time PCR, for research purposes only.

Purchase of Digital PCR and/or Single-Cell NGS Sample Preparation products (the "Products") from Bio-Rad Laboratories is subject to Bio-Rad Laboratories, Inc. Standard Terms and Conditions of Sale, which can be accessed at https://www.bio-rad.com/en-us/terms-conditions. Unless we expressly state otherwise in additional Terms and Conditions, no rights are granted for you to distribute or resell the Products. Unless we expressly state otherwise in additional Terms and Conditions, no rights are granted for the development or commercialization of diagnostic assays for use with the Products without a license from Bio-Rad. The Products and/or their use are covered by U.S. and foreign patents and/or pending patent applications owned by or under license to Bio-Rad Laboratories, Inc. See https://www.bio-rad.com/en-us/trademarks.



Bio-Rad Laboratories. Inc.

Life Science Group Website bio-rad.com USA 1 800 424 6723 Australia 61 2 9914 2800 Austria 00 800 00 24 67 23 Belgium 00 800 00 24 67 23 Brazil 4003 0399 Canada 1 905 364 3435 China 86 21 6169 8500 Czech Republic 00 800 00 24 67 23 Denmark 00 800 00 24 67 23 Finland 00 800 00 24 67 23 France 00 800 00 24 67 23 Germany 00 800 00 24 67 23 Hong Kong 852 2789 3300 Hungary 00 800 00 24 67 23 India 91 124 4029300 Israel 0 3 9636050 Italy 00 800 00 24 67 23 Japan 81 3 6361 7000 Korea 82 2 3473 4460 Luxembourg 00 800 00 24 67 23 Mexico 52 555 488 7670 The Netherlands 00 800 00 24 67 23 Poland 00 800 00 24 67 23 Poland 00 800 00 24 67 23 Singapore 65 6415 3188 South Africa 00 800 00 24 67 23 Spain 00 800 00 24 67 23 Sweden 00 800 00 24 67 23 Switzerland 00 800 00 24 67 23 Talwan 886 2 2578 7189 Thailand 66 2 651 8311 United Arab Emirates 36 1 459 6150 United Kingdom 00 800 00 24 67 23

Bulletin 6090 Ver H US/EG 21-0813 1121 Sig 0121

