Quantabio

PRODUCT GUIDE

Sample Preparation | Reverse Transcription | PCR & qPCR | Genotyping | microRNA Profiling | NGS

ABOUT US

Quantabio is a leading provider of advanced DNA and RNA amplification reagents for the most demanding molecular testing applications in applied, translational and life science research. The Quantabio team leverages decades of experience in developing pioneering amplification technologies to deliver cutting-edge products to researchers focused on critical cloning, PCR, qPCR and Next-Generation Sequencing (NGS) based applications. Based in Beverly, Mass., Quantabio offers a growing portfolio of products through its international sales operations, as well as a global network of distributors and commercial service providers. For more information, please visit **www.quantabio.com**.

QUANTABIO REAGENT TECHNOLOGIES

Manufacturing Excellence

- ISO 13485 quality certified
- Ultrapure, performance-engineered enzymes
- Ultralow residual host *E. coli* DNA

Engineered Stability

- Stringent enzyme activation control with AccuStart technology
- Reaction setup and multi-day storage at ambient temperature
- Impervious to repetitive freeze-thaw

Formulated for Quantitative Real-Time PCR

- Optimized 1-tube reagents minimize pipetting steps and improve accuracy
- Supports efficient vortex mixing and eliminates error-causing persistent bubbles
- Inert AccuVue plate loading dye provides visual confirmation of reaction assembly

Tough-Tested

- ToughMix reagents withstand a broad spectrum of PCR inhibitors
- Reliable assay performance with challenging starting materials and crude extracts

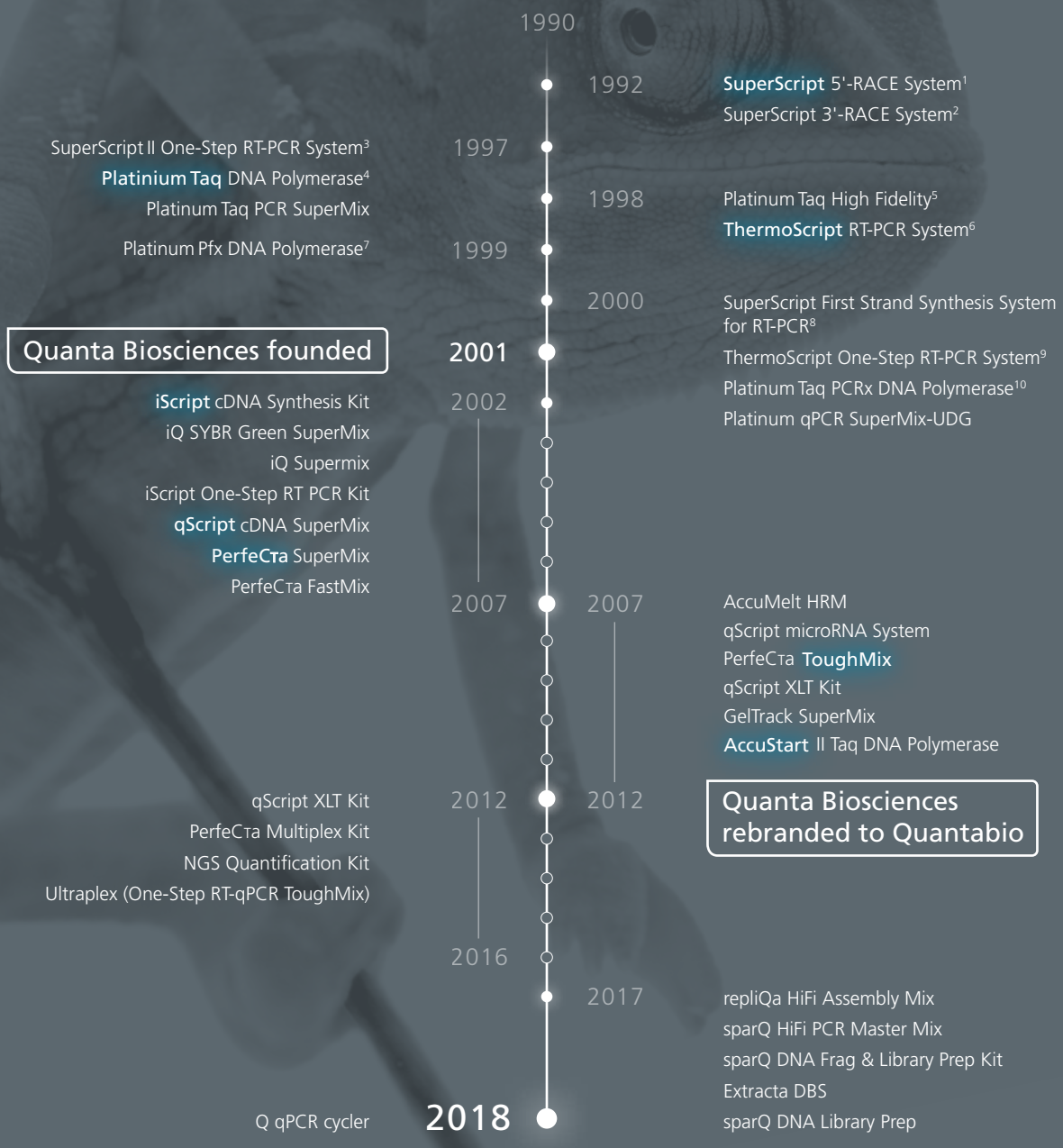
Optimized to improve sequencing performance & economics

- Comprehensive solutions for DNA fragmentation, library preparation, amplification and quantification
- Novel formulations streamline NGS workflows reducing total turn-around-time
- Proprietary enzymes & buffer compositions improve library yields and sequencing results from low inputs

Legacy of Innovation

Quantabio has a legacy of pioneering market leading PCR & qPCR reagents including Invitrogen's SuperScript One-Step RT-PCR Kits, Platinum Taq, Bio-Rad's iScript and iQ/iTaq Supermixes. Most recently the brand has begun providing leading reagent solutions for cloning and NGS.

QUANTABIO HISTORY TIMELINE



CONTENT

CORE TECHNOLOGIES

RT-qPCR is a powerful molecular biology technologies.

RT-qPCR

Reproducible

Quantabio kits define experimental reproducibility through multiple proprietary technologies: ultra low *E. coli* DNA, low foam, and enhanced stability. Due to our exacting lot-to-lot consistency standards and innovative 1-tube formulations that minimize pipetting, our reagent technologies provide highly consistent results. Patented additives actively reduce intra-assay variability and withstand repetitive cycles of freeze-thaw to deliver assay reliability.

RT-qPCR

ToughMix

PCR inhibitors are common in crude samples and readily compromise assay performance. Use of Quantabio TOUGH-tested ToughMix reagents results in enhanced PCR performance with crude or contaminated samples. The advanced ToughMix buffer technology is engineered to withstand several types of PCR-inhibition, providing robust and reliable results from a variety of starting materials and purification methods.

RT-qPCR

qScript

We put the "Q" in RT-qPCR with our advanced reverse transcriptase technology that is synonymous with maximum yield and sensitivity. qScript first-strand cDNA synthesis reagents are rigorously optimized to provide sensitive and reliable detection of low abundance RNA for qPCR assays. The broad, linear dynamic range of input RNA (10 pg - 1 µg) provides reliableassay sensitivity for robust gene expression analysis.

tool that represents the Quantabio core

RT-qPCR

Perfecting qPCR

Our PerfeCTa real-time quantitative PCR reagents are rigorously optimized; all-in-one reagents that dramatically simplify reaction setup and contain patented technologies to actively reduce assay variance. Our robust, ultrapure antibody hot start AccuStart technology drives precise target amplification with the absolute maximum limit of detection sensitivity.

RT-qPCR

Customization

Our proprietary formulation processes allow us to rapidly configure customized reagent solutions according to client specific needs. Whether it's a defined lot size for a large scale project, a packaging or fill volume requirement to suit a particular workflow, or modified composition to fine tune assay performance, Quantabio has the flexible responsiveness to realize your custom needs.

RT-qPCR

Reliable

Have you been negatively impacted by a backorder delay, a change in lot performance, or a lapse in technical support? Quantabio is your trusted reagent supply partner. We pride ourselves on manufacturing quality excellence and an industry-leading technology portfolio.

1.0 Sample Preparation

Extracta DNA Prep for PCR

qPCR-grade genomic DNA template in record time

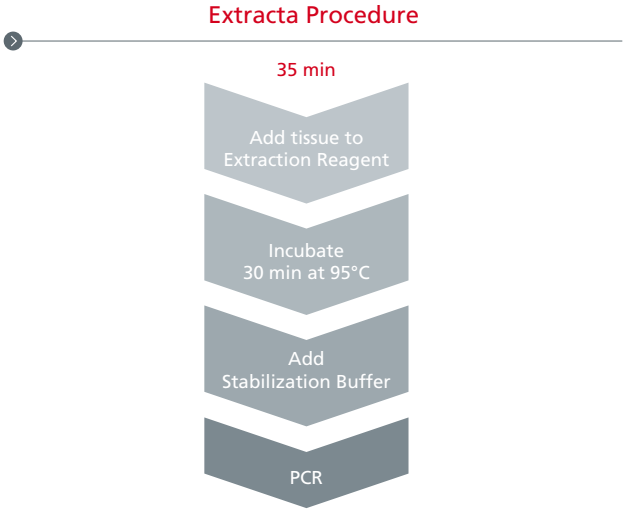
Extracta DNA Prep is an entirely reagent-based system for extracting and stabilizing template DNA from a variety of biological starting materials for sensitive downstream applications such as PCR, qPCR and HRM analysis

FEATURES AND BENEFITS:

- Simple, reagent-based system requires minimal technical skill
- Incubation step can be carried out in 96-well PCR plates or tubes using a standard DNA thermal cycler
- Compatible with a wide-range of clinical specimens, plant and animal tissues, and environmental samples
- Optional stabilization buffer allows for extended storage of extracted DNA templates

DESCRIPTION:

Extracta DNA Prep for PCR is a two-component reagent kit for rapid extraction of PCR-ready genomic DNA from a variety of tissues. Samples are processed in less than 30 minutes with minimal hands-on time and technical skill. Extracted genomic DNA is suitable for sensitive downstream PCR applications including end-point PCR, High Resolution Melt Analysis (HRM) and quantitative real-time PCR (qPCR) without requiring any additional clean-up. In addition, the extracted DNA may be used in multiplexed PCR applications such as transgene or knock-out analyses. Tissue extractions can be done in tubes, plates or deep-well blocks to allow for adaptation to workflow and automation on liquid-handling workstations.



ORDER INFO

Product Name	Quantabio Catalog Number	Size
Extracta DNA Prep for PCR - 2.5 ml	95091-002	2.5 ml
Extracta DNA Prep for PCR - 25 ml	95091-025	25 ml
Extracta DNA Prep for PCR - 250 ml	95091-250	250 ml

Extracta DBS

PCR-ready genomic DNA from dried blood spots

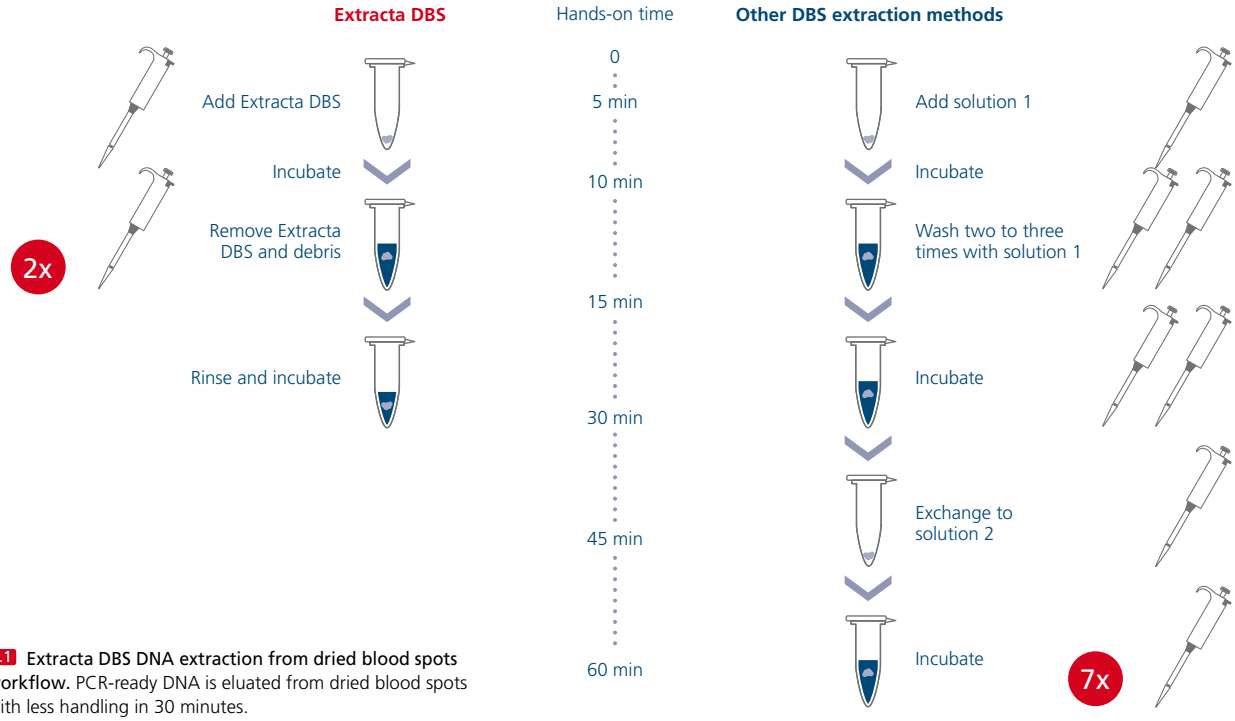
FEATURES AND BENEFITS:

- Optimized for DNA extraction from dried blood spot punches
- Single reagent for PCR-ready DNA in less than 30 minutes
- Maximized assay sensitivity, lower Cq values, when combined with Quantabio ToughMix
- Compatible with high-throughput automation for PCR, qPCR and NGS applications

DESCRIPTION:

Extracta DBS is a ready-to-use DNA extraction reagent for rapid and efficient recovery of PCR-ready DNA from dried blood spots (DBS) on Guthrie cards or Whatman 903 filter paper. This patented single-solution process produces DNA eluates that are substantially free of PCR inhibitors and compatible with a variety of end-point PCR, real-time PCR and Next Generation Sequencing (NGS) or Sanger Sequencing (1-3) reagents. Application of Extracta DBS with PerfeCta qPCR ToughMix or PerfeCta MultiPlex qPCR ToughMix enables accurate and reproducible quantification of DNA sequences in blood using TaqMan hydrolysis probe real-time qPCR.

Fast, single solution extraction workflow in 30 min



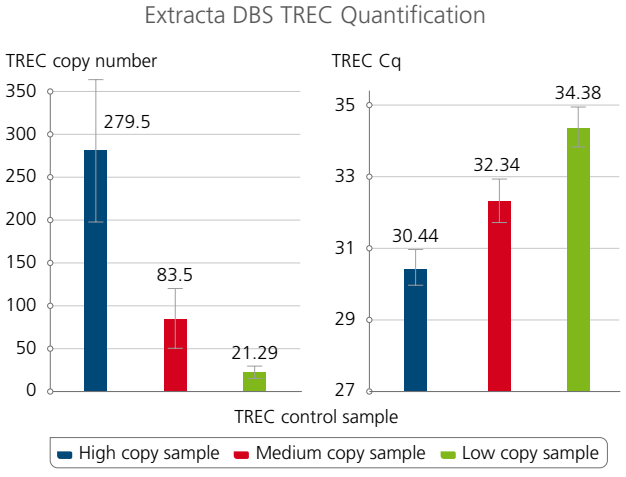
1.1 **Extracta DBS DNA extraction from dried blood spots workflow.** PCR-ready DNA is eluated from dried blood spots with less handling in 30 minutes.

Combine with ToughMix and maximize qPCR assay sensitivity

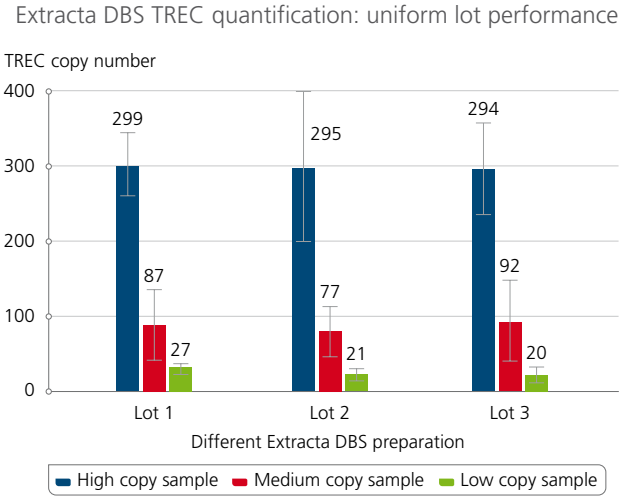
Extracta DBS is the perfect match with Quantabio ToughMix for sensitive and precise target quantification. The crude extraction combined with ToughMix, a Quantabio master mix that is tolerant to common PCR inhibitors, results in higher DNA yields independent of DNA sample inputs and qualities to enable accurate detection and high sensitivity even with low copy targets.

Reliable, Consistent Lot-to-Lot Performance

Low amounts and quality of DNA recovered from dried blood spots commonly restrict the utilization of DNA. To overcome these limitations, Extracta DBS increases the yield and quality allowing for efficient and reliable recovery of DNA from dried blood spots. Manufactured under stringent ISO 13485 standards, Extracta DBS ensures uniform lot-to-lot performance resulting in reliable reproducibility in combination with Quantabio ToughMix.



1.2 Illustrates the results of a T-cell Receptor Excision Circles (TREC) assay using Extracta DBS and PerfeCta ToughMix. Samples were generated using dried blood spot punches following the Extracta DBS protocol and used subsequently for quantification of T-cell Receptor Excision Circles. The samples are representatives of High, Medium and Low TREC copy numbers along with the corresponding Cq values.



1.3 This figure demonstrates consistent lot-to-lot performance in a TREC quantification assay using genomic DNA extracted from dried blood spots. Lot-to-lot performance was tested for High, Medium and Low copy number TREC samples. The results highlight the reliability and reproducibility across various product lots which are attributed to Quantabio's high manufacturing and production standards under ISO 13485.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
Extracta DBS, 10 ml	95171-010	10 ml
Extracta DBS, 500 ml	95171-500	500 ml
Related Products		
PerfeCta qPCR ToughMix - 250 R	95112-250	250 x 20 µl rxns (2 x 1.25 ml)
PerfeCta qPCR Multiplex ToughMix - 250 R	95112-250	250 x 20 µl rxns (2 x 1.25 ml)

Quantabio ToughMixes are also available with different concentrations of ROX and in larger reaction sizes.

5PRIME Phase Lock Gel

Phase Lock Gel simplifies organic extraction of nucleic acid (NA) template and improves safety

Organic extraction methods are cost-effective and result in the highest yields of nucleic acid template but are not user friendly and involve hazardous chemicals

FEATURES AND BENEFITS:

- Inert gel creates a physical barrier between the precious aqueous phase and harmful organic phase/interphase material
- Simplifies recovery of pure NA without the need for advanced pipetting skill

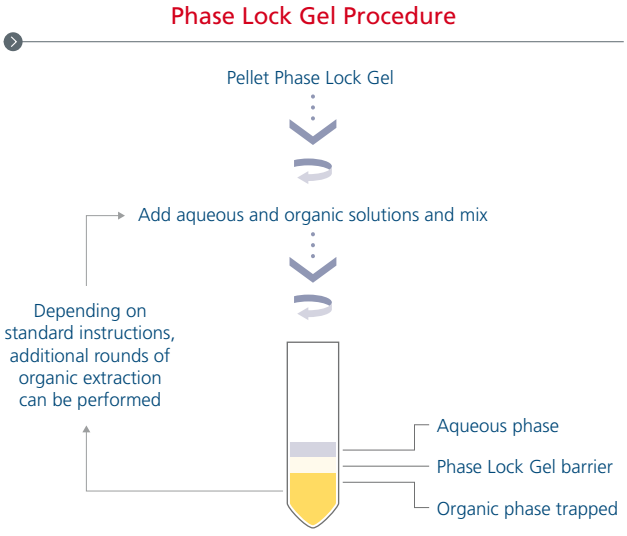
DESCRIPTION:

Phase Lock Gel (PLG) is a unique product that eliminates inter-phase-protein contamination during phenol extraction of DNA or RNA. PLG reduces hands-on time and improves nucleic acid recovery. PLG migrates under centrifugal force to form a tight seal between the aqueous and the organic phase. The organic phase and the interphase materials are effectively trapped in or below the barrier. The stable barrier enables a complete and easy transfer of the aqueous, nucleic acid containing upper phase to a fresh tube.

The benefits are increased yields by up to 30%, better protection from exposure to hazardous compounds and no risk of sample contamination with interphase debris. PLG can be adapted to virtually any protocol requiring extraction of an aqueous sample with phenol and/or chloroform. For convenience, PLG is provided aliquoted into standard centrifuge tubes of various sizes.

Phase Lock Gel Light and Heavy Applications and Compatibilities

For optimal phase separation the composition of the aqueous phase, the organic phase and PLG must be compatible as the ability of PLG to separate the phases depends on matching the differences in density between aqueous and organic media. Besides general differences of the organic phase due to the starting material, density is also influenced by salt and protein concentration in the aqueous phase. To ensure compatibility, PLG comes in 2 density formulations, Heavy (H) and Light (L). Choose the formulation that fits your specific application from the table next page:



Phase Lock Gel, Density Selection Chart

Aqueous Phase	Organic Phase			
	PCI	CI	H_2O or Buffer saturated PC	H_2O or Buffer saturated Phenol
< 0.5 M NaCl	L, H	L, H	L, H	L
< 1 mg/ml BSA	L, H	L, H	L, H	L
Cleared bacterial lysate	H	H	H	–
Plasmid DNA homogenates	H	H	H	–
Tissue homogenates	L, H	L, H	L, H	L
Genomic DNA isolation	L, H	L, H	L, H	L
RNA isolation	H	H	H	–

PCI = 25:24:1 Phenol : Chloroform : Isoamyl Alcohol
PC = 1:1 Phenol : Chloroform
CI = 24:1 Chloroform : Isoamyl Alcohol
– = Conditions unsuitable for Phase Lock Gels

ORDER INFO

Product Name	Quantabio Catalog Number	Size
Phase Lock Gel Heavy 2 ml - 200 Tubes	2302830	200 tubes
Phase Lock Gel Light 2 ml - 200 Tubes	2302820	200 tubes

Reverse Transcription 2.0

Quantabio qScript reverse transcriptase technologies set the standard for simplicity, reproducibility, and broad linear dynamic range for quantitative and conventional RT-PCR applications.

qScript cDNA synthesis reagents provide highly sensitive first-strand cDNA synthesis in a variety of easy-to-use reagent configurations for RT-PCR and RT-qPCR.

Ultrapure, performance-engineered M-MLV reverse transcriptases are pre-blended with ribonuclease (RNase) inhibitor protein in rigorously optimized 1-tube SuperMix formulations and separate component kits to suit specific assay designs and workflow preferences.

First-Strand cDNA Synthesis 2.1

PRODUCT OVERVIEW

	Superior Performance	Reliable Simplicity	Exceptional Value	User-Defined Priming	Small ncRNA
Kit	**qScript XLT SuperMix**	**qScript cDNA SuperMix**	**qScript cDNA Synthesis Kit**	**qScript Flex cDNA Kit**	**qScript microRNA cDNA Synthesis Kit**
cDNA Yield & Sensitivity	++++	+++	++	++	+++
Reagent Components	1	1	2	5	3
Total Reaction Time	70 min	40 min	40 min	60-90 min	50–90 min
RT Priming Method	qPCR-optimized oligo(dT) and random hexamer blend	qPCR-optimized oligo(dT) and random hexamer blend	qPCR-optimized oligo(dT) and random hexamer blend	Oligo(dT), random hexamer, or gene-specific (GSP)	PAP with Proprietary Adapter Oligo(dT)
RNA Input (Linear Range)	1 pg – 2 µg	10 pg – 1 µg	10 pg – 1 µg	10 pg – 1 µg	10 pg – 1 µg
Amplicon Length	<1 kb	<1 kb	<1 kb	12+ kb	<1 kb

qScript cDNA SuperMix/qScript XLT cDNA SuperMix

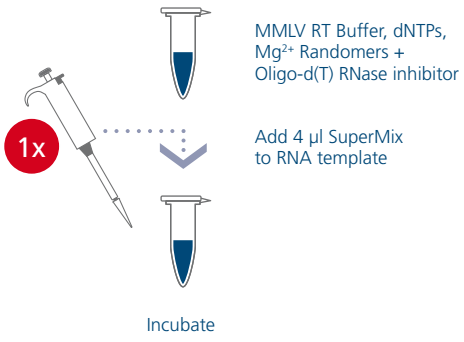
Superior cDNA synthesis in a single step

FEATURES AND BENEFITS:

- 5x concentrated SuperMix maximizes input volume with dilute samples of total RNA
- Broad linear dynamic range with limiting (10 pg) or plentiful samples of total RNA
- Pre-blended with ribonuclease inhibitor protein (RIP) and an optimized blend of random hexamer and oligo(dT) primers to ensure accurate representation of 5' and 3' sequences

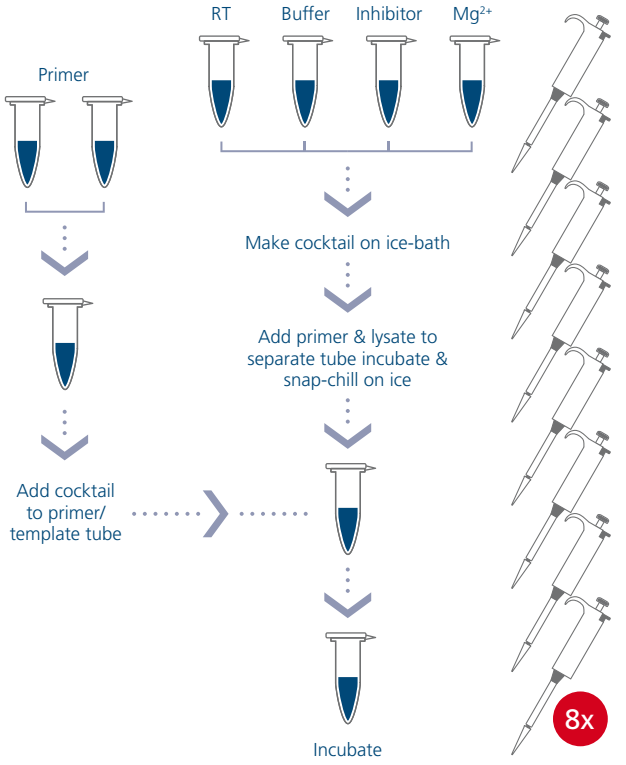
qScript cDNA SuperMix

Stabilized 1-tube SuperMixes simplify reaction assembly and minimize risk of pipetting error



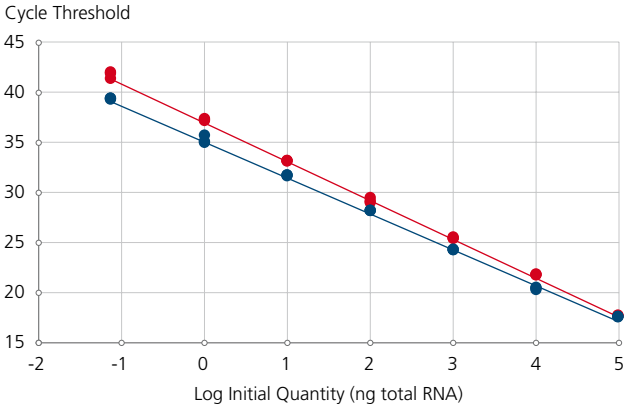
- 1 Tube
- 1 Pipetting Step
- 40 Minutes (70 min for qScript XLT Supermix)

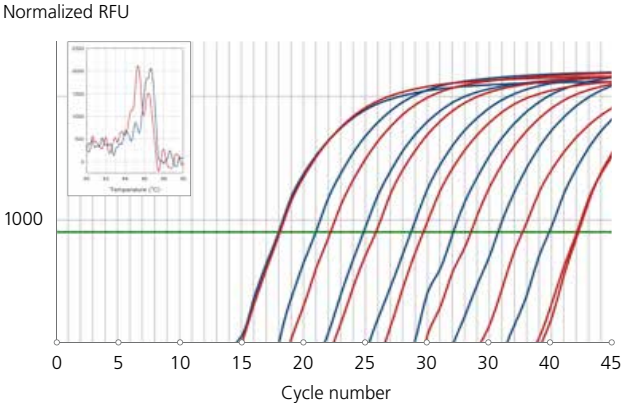
Other cDNA Kits



2.1 qScript cDNA SuperMix (left) **includes all necessary components in a single tube – just add RNA and go!** Compare this to competitor options (right) that introduce numerous opportunities for error.

qScript cDNA SuperMix Delivers Higher Yields, Improved Representation of Low Abundance Genes and Superior Linear Dynamic Range

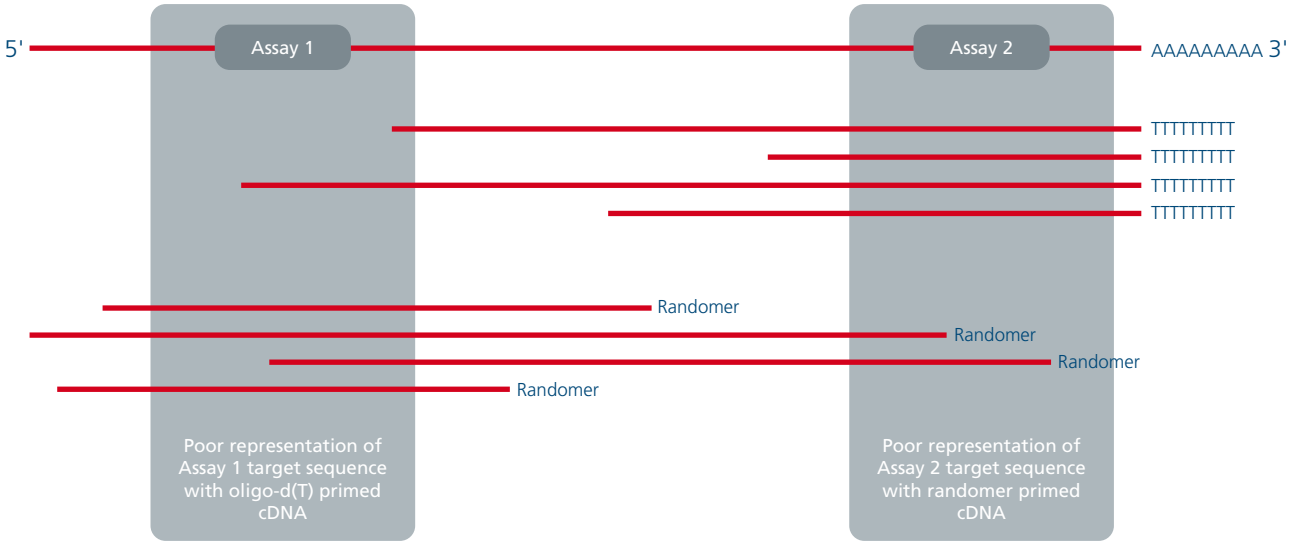




	Slope	Corr.	PCR Eff.	Sensitivity
qScript cDNA SuperMix	-3.625	0.998	88.7%	50 fg
Competitor S	-3.941	0.999	79.4%	500 fg

2.2 Increasing amounts of HeLa total RNA (1 pg – 1 µg) were reverse transcribed using qScript cDNA SuperMix in parallel with another leading supplier kit, according to supplied protocols.

Mix of random primers and oligo dT prevents bias to the 5' or 3' ends of the target



qScript cDNA Synthesis Kit

Economical 2-component kit ideally suited for high throughput gene-expression studies

FEATURES AND BENEFITS:

- Sensitive first-strand cDNA synthesis of RNA sequences ≤1kb for quantitative and conventional two-step RT-PCR
- Broad linear dynamic range with limiting (10 pg) or plentiful samples of total RNA
- Pre-blended with ribonuclease inhibitor protein (RIP) and an optimized blend of random hexamer and oligo(dT) primers to ensure accurate representation of 5' and 3' sequences

qScript Flex cDNA Synthesis Kit

Highly flexible first-strand synthesis system suitable for large target RNA sequences

FEATURES AND BENEFITS:

- User choice of RT priming method; oligo(dT), random hexamers, or GSP
- Highly sensitive first-strand cDNA synthesis of large RNA sequences for quantitative and conventional two-step RT-PCR
- Broad linear dynamic range with limiting (10 pg) or plentiful total RNA samples
- Maximize cDNA yield with proprietary Priming Enhancer additive

ORDER INFO

Product Name	Quantabio Catalog Number	Size
qScript cDNA SuperMix - 25 R	95048-025	25 rxns
qScript cDNA SuperMix - 100 R	95048-100	100 rxns
qScript cDNA SuperMix - 500 R	95048-500	500 rxns
qScript XLT cDNA SuperMix - 25 R	95161-025	25 rxns
qScript XLT cDNA SuperMix - 100 R	95161-100	100 rxns
qScript XLT cDNA SuperMix - 500 R	95161-500	500 rxns
qScript cDNA Synthesis Kit - 25 R	95047-025	25 rxns
qScript cDNA Synthesis Kit - 100 R	95047-100	100 rxns
qScript cDNA Synthesis Kit - 500 R	95047-500	500 rxns
qScript Flex cDNA Kit - 25 R	95049-025	25 rxns
qScript Flex cDNA Kit - 100 R	95049-100	100 rxns

Reverse Transcription PCR (RT-PCR) 2.2

PRODUCT OVERVIEW

	Conventional One-Step RT-PCR	Tough-Tested MultiPlex One-Step RT-qPCR	Tough-Tested One-Step Probe-based RT-qPCR	One-Step Probe-based RT-qPCR	One-Step SYBR-based RT-qPCR
Kit	**qScript XLT One-Step RT-PCR Kit**	**UltraPlex One-Step ToughMix**	**qScript XLT One-Step RT-qPCR ToughMix**	**qScript One-Step RT-qPCR Kit**	**qScript One-Step SYBR Green RT-qRT PCR**
Detection Chemistry	N/A	Hydrolysis Probes	Hydrolysis Probes	Hydrolysis Probes	SYBR Green I dye
Sensitivity	+++	++++	+++	++	++
Multiplex Compatibility	N/A	>4	<4	<3	No
Reagent Components	2	1	1	2	2
RNA Input (Linear Range)	1 pg – 1 µg	• 1 pg to 100 ng total RNA; • 10 fg to 10 ng poly A(+) RNA; • 10 to 1x10^8 copies viral RNA			
Amplicon Length	4+ kb	<1 kb	<1 kb	<1 kb	<1 kb

Conventional RT-PCR

qScript XLT One-Step RT-PCR Kit

Tough-tested One-Step Reverse Transcriptase PCR (RT-PCR) in a simplified, 2-component reagent system

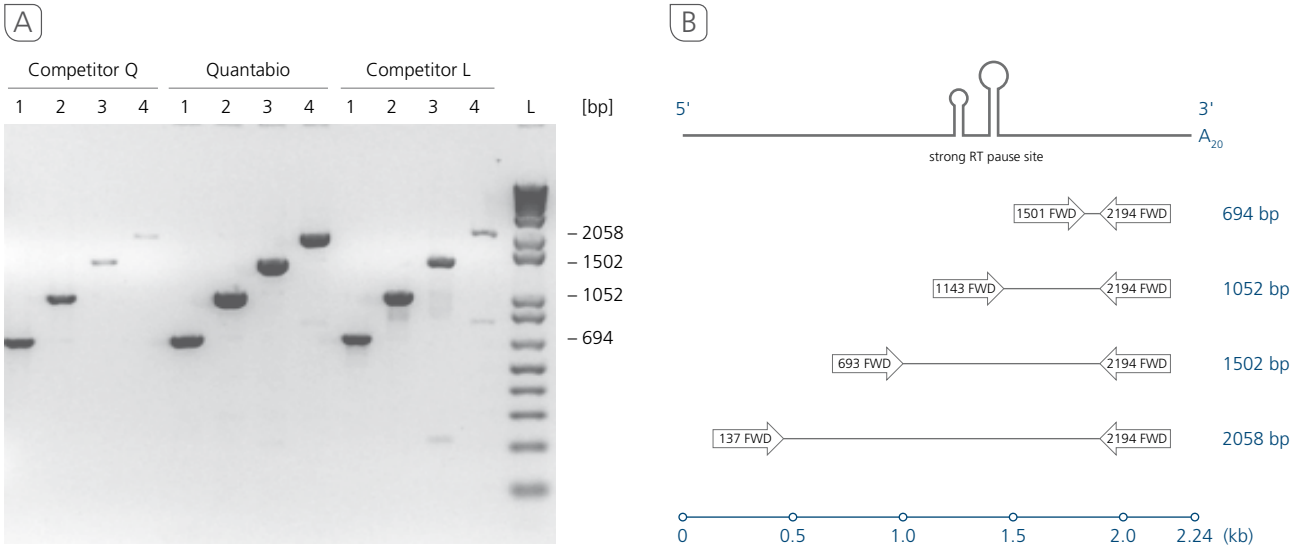
The qScript XLT One-Step RT-PCR kit provides highly sensitive detection of large, complex RNA in challenging starting materials supporting high-fidelity downstream applications

FEATURES AND BENEFITS:

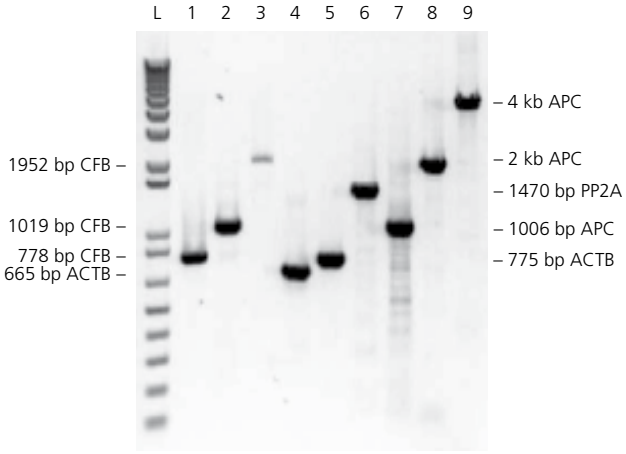
- ToughMix reagent technology withstands PCR inhibitors commonly found in plant and animal tissues, environmental sources, clinical specimens and complex food matrices
- Optional GelTrack dye streamlines workflow for gel electrophoresis
- Temperature stabilized for support reaction assembly at convenient ambient room temperatures
- Preblended with ribonuclease inhibitor protein to preserve RNA integrity during incubation
- 3'-exonuclease proof-reading polymerase supports high-fidelity downstream applications
- Suitable for TA subcloning large RNA sequences exceeding 4 kb in length

DESCRIPTION:

The qScript XLT One-Step RT-PCR Kit is a convenient and highly sensitive 2-reagent system for amplification of complex RNA templates exceeding 4 kb in length. Both enzyme incubation sequences are carried out in the same reaction mixture without opening between procedures. Advanced qScript XLT reverse transcriptase mutant possesses elevated temperature stability and improved template binding affinity for large complex RNA templates. Supports a wide-range of challenging starting materials with PCR-inhibitor neutralizing ToughMix additives and delivers consistent, reliable assay performance. Ultrapure AccuStart II hot-start Taq DNA polymerase with 3'-exonuclease proof-reading activity provides stringent activation control for sensitive and precise target amplification.



2.3 One-Step RT-PCR of varying length amplicons from 2.2 kb TcR in vitro transcript RNA. Each kit was used according to the manufacturer's recommended procedure in 20 µl reaction volumes containing 200 µM each primer and 1x 10^5 copies of an in vitro synthesized run-off transcript for the tetracyclin resistance gene (TcR), produced using T7 RNA polymerase. Following first-strand synthesis and activation of the hot-start Taq polymerase, all reactions were amplified for 30 cycles of 94°C, 15 s; 60°C, 20 s; 72°C, 2 min followed by a final hold of 5 min at 72°C. 1/5th of each reaction was analyzed on a 0.8% agarose, 0.5x TBE gel conatining 0.25 mg/ml ethidium bromide.



2.4 One-Step RT-PCR of varying length fragments from HeLa cell total RNA. RT-PCR program: 48°C 20 min; 94°C, 3 min; 94°C, 15 s; 60°C 15 s; 68°C, 2 min; 35 cycles. ACTB = 2 ng HeLa total RNA, all others 20 ng HeLa total RNA Load 5 µl of 20 µl rxn on 0.8% gel.

CFB = Complement Factor B
PP2A = protein phosphatase 2A
ACTB = β-actin
APC = adenomatous polyposis coli)

ORDER INFO

Product Name	Quantabio Catalog Number	Size
qScript XLT One-Step RT-PCR Kit - 20 R	95143-020	20 x 25 µl rxns
qScript XLT One-Step RT-PCR Kit - 200 R	95143-200	200 x 25 µl rxns

Quantitative RT-qPCR

qScript One-Step SYBR Green RT-qPCR

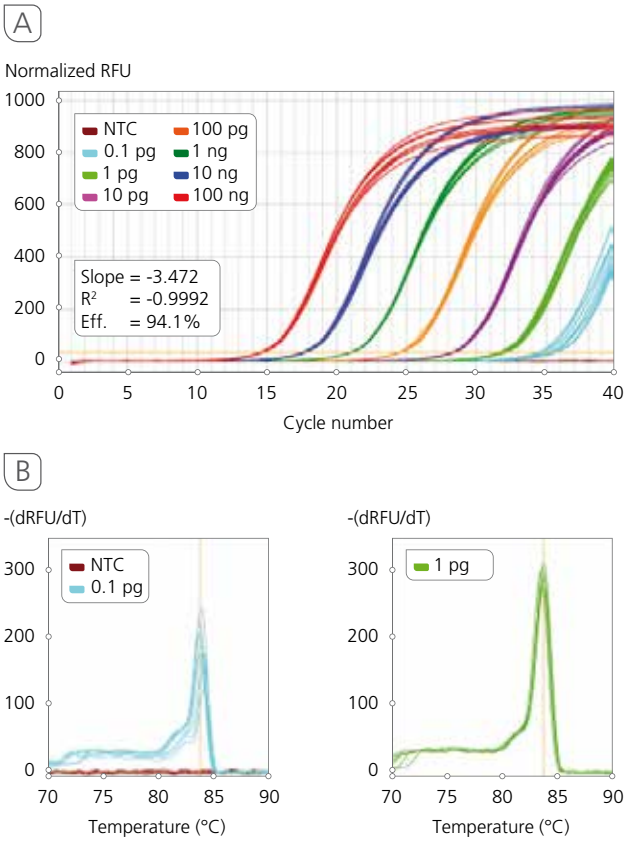
Sensitive, dye-based RNA quantification with gene specific primers in a single, seamless reaction mixture without opening the tube prior to PCR

FEATURES AND BENEFITS:

- One-step reaction minimizes opportunity for pipetting error
- Robust, specific amplification
- AccuStart hot start mAb technology

DESCRIPTION:

The qScript One-Step SYBR Green RT-qPCR Kit is a convenient and highly sensitive solution for quantitative RT-PCR of RNA templates (RT-qPCR) using SYBR Green I dye detection and gene-specific primers. cDNA synthesis and PCR amplification are carried out in the same tube without opening between procedures. The system has been optimized to deliver maximum RT-PCR efficiency, sensitivity and specificity. The proprietary reaction buffer has been specifically formulated to maximize activities of both reverse transcriptase and Taq DNA polymerase while minimizing the potential for primer-dimer and other non-specific PCR artifacts. The kit is compatible with both fast and standard qPCR cycling protocols. Highly specific amplification is essential for successful RT-qPCR with SYBR Green I technology, since this dye binds to any dsDNA generated during amplification. AccuStart Taq DNA polymerase contains monoclonal antibodies that bind to the polymerase and keep it inactive prior to the initial PCR denaturation step. Upon heat activation at 95°C, the antibodies denature irreversibly, releasing fully active, unmodified Taq DNA polymerase.



2.5 One-Step SYBR Green RT-qPCR with broad dynamic range, high sensitivity and high specificity. A 202 bp fragment of glyceraldehyde-3-phosphate dehydrogenase (GAPD) mRNA was amplified from log-fold serial dilutions of HeLa cell total RNA (100 ng to 0.1 pg). Eight replicate reactions for each RNA quantity, and the no template control (NTC) were carried out in 25 µl volumes with the qScript One-Step SYBR Green RT-qPCR Kit and 200 nM each GAPD specific primers (PrimerBank ID 7669492a2, Wang, X. and Seed, **B** (2003) NAR 31(24): e154; pp.1-8). Reactions were assembled on ice, transferred to a MyiQ™ real-time detection system (Bio-Rad Laboratories), and incubated for 5 min at 50°C followed by 2 min at 95°C. PCR cycling was for 40 cycles of 3 s, 95°C; 30 s, 60°C. Immediately following PCR cycling the block temperature was ramped from 60°C to 95°C and melt curve data was collected. Panel A) Amplification plots and standard curve regression analysis. Panel B) Dissociation results (melt curve) for NTC, 0.1 pg and 1 pg reactions.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
qScript One-Step SYBR Green RT-qPCR Kit for iQ - 50 R	95086-050	50 x 50 µl rxns
qScript One-Step SYBR Green RT-qPCR Kit for iQ - 200 R	95086-200	200 x 50 µl rxns
qScript One-Step SYBR Green RT-qPCR Kit - 50 R	95087-050	50 x 50 µl rxns
qScript One-Step SYBR Green RT-qPCR Kit - 200 R	95087-200	200 x 50 µl rxns
qScript One-Step SYBR Green RT-qPCR Kit, ROX - 50 R	95088-050	50 x 50 µl rxns
qScript One-Step SYBR Green RT-qPCR Kit, ROX - 200 R	95088-200	200 x 50 µl rxns
qScript One-Step SYBR Green RT-qPCR Kit, Low ROX - 50 R	95089-050	50 x 50 µl rxns
qScript One-Step SYBR Green RT-qPCR Kit, Low ROX - 200 R	95089-200	200 x 50 µl rxns

qScript XLT One-Step RT-qPCR ToughMix

Robust, inhibitor-resistant RT-qPCR, maximum yields for superior performance

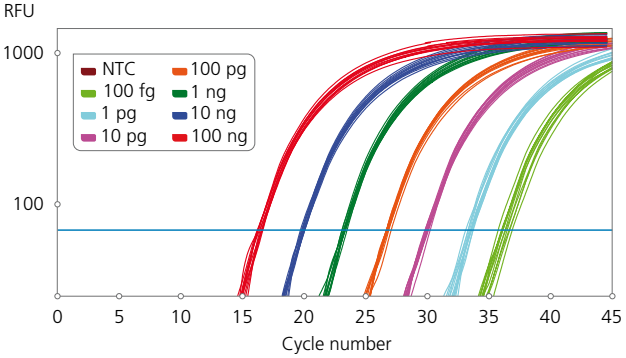
FEATURES AND BENEFITS:

- ToughMix reagent technology neutralizes a broad spectrum of PCR inhibitors common in plant and animal tissues, environmental sources, clinical specimens and complex food matrices
- Superior assay sensitivity and specificity with ultrapure AccuStart II enzyme technology – maximum-yielding Taq DNA polymerase mutant controlled by stringent, multi-epitope antibody hot start
- Inert AccuVue plate loading dye simplifies reaction setup and provides instant visual cue of reagent addition and mixing
- Supports efficient vortex mixing with proprietary anti-foaming formulation
- Flexible – supports for both fast and standard thermal cycling conditions

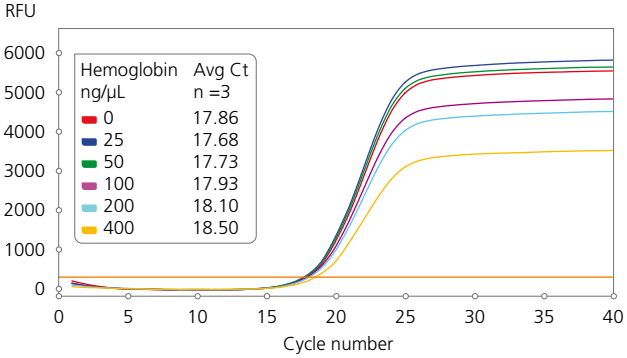
DESCRIPTION:

qScript XLT One-Step RT-qPCR ToughMix is a ready-to-use, highly sensitive master mix for reverse transcription quantitative PCR (RT-qPCR) of RNA templates using hybridization probe detection chemistries. Advanced qScript XLT reverse transcriptase mutant possesses elevated temperature stability and improved template binding affinity for large complex RNA templates. Supports a wide-range of challenging starting materials with PCR-inhibitor neutralizing ToughMix additives and delivers consistent, reliable assay performance.

Sequential temperature incubations are performed to the same reaction mixture without opening the tube. The proprietary one-step reaction buffer has been specifically formulated to maximize activity of each enzyme while minimizing the potential for primer-dimer and other non-specific PCR artifacts. Inert AccuVue plate loading dye simplifies reaction assembly and provides instant visual confirmation of reagent addition and mixing.



2.6 Broad linear dynamic range, low Limit of Detection.



2.7 Enables performance in the presence of inhibitors.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
qScript XLT One-Step RT-qPCR ToughMix - 100 R	95132-100	100 x 20 µl rxns
qScript XLT One-Step RT-qPCR ToughMix - 500 R	95132-500	500 x 20 µl rxns
qScript XLT One-Step RT-qPCR ToughMix - 2000 R	95132-02K	2000 x 20 µl rxns
qScript XLT One-Step RT-qPCR ToughMix, ROX - 100 R	95133-100	100 x 20 µl rxns
qScript XLT One-Step RT-qPCR ToughMix, ROX - 500 R	95133-500	500 x 20 µl rxns
qScript XLT One-Step RT-qPCR ToughMix, ROX - 2000 R	95133-02K	2000 x 20 µl rxns
qScript XLT One-Step RT-qPCR ToughMix, Low ROX - 100 R	95134-100	100 x 20 µl rxns
qScript XLT One-Step RT-qPCR ToughMix, Low ROX - 500 R	95134-500	500 x 20 µl rxns

qScript One-Step RT-qPCR

Sensitive RNA quantification with probe-based detection chemistries in a single, seamless reaction mixture without opening the tube prior to PCR

FEATURES AND BENEFITS:

- Simplified 2-reagent system supports user-friendly reaction setup at ambient temperature
- Highly sensitive RNA detection with performance engineered, qScript RNase H(+) M-MLV reverse transcriptase mutant
- Superior assay sensitivity and specificity with AccuStart hot start enzyme technology
- Compatible with either fast or standard thermal cycling conditions

DESCRIPTION:

The qScript One-Step RT-qPCR kit is a convenient, 2-component reagent system that supports highly sensitive one-step real-time PCR detection assays of RNA templates (RT-qPCR) and is compatible with all dual-labeled (hydrolysis) probe chemistries. First-strand cDNA synthesis and PCR amplification are carried out in the same tube without opening between procedures. Specifically formulated to maximize activities of both reverse transcriptase and Taq DNA polymerase while minimizing the potential for primer-dimer and other non-specific PCR artifacts; this proprietary one-step formulation has been optimized to deliver maximum RT-PCR efficiency, sensitivity, and specificity, enabling unbiased co-amplification of low copy transcripts in the presence of higher copy reference genes.

Highly specific amplification is crucial to successful RT-qPCR as non-specific product(s) can compete for amplification of the target sequence and impair PCR efficiency. A key component of this kit is AccuStart Taq DNA polymerase which contains monoclonal antibodies that bind to the polymerase and keep it inactive prior to the initial PCR denaturation step. Upon heat activation at 95°C, the antibodies denature irreversibly, releasing fully active, unmodified Taq DNA polymerase.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
qScript One-Step RT-qPCR Kit - 50 R	95057-050	50 x 50 µl rxns
qScript One-Step RT-qPCR Kit - 200 R	95057-200	200 x 50 µl rxns
qScript One-Step RT-qPCR Kit, ROX - 50 R	95058-050	50 x 50 µl rxns
qScript One-Step RT-qPCR Kit, ROX - 200 R	95058-200	200 x 50 µl rxns
qScript One-Step RT-qPCR Kit, ROX - 1000 R	95058-01K	1000 x 50 µl rxns
qScript One-Step RT-qPCR Kit, Low ROX - 50 R	95059-050	50 x 50 µl rxns
qScript One-Step RT-qPCR Kit, Low ROX - 200 R	95059-200	200 x 50 µl rxns

UltraPlex One-Step ToughMix

Up to 5-target multiplex, inhibitor-resistant RT-qPCR, maximum yields for superior performance

FEATURES AND BENEFITS:

- 4x concentrated SuperMix reagent supports increased sample input volume, improving flexibility with extremely low yield templates (1 pg total RNA)
- ToughMix reagent technology neutralizes a broad spectrum of PCR inhibitors common in plant and animal tissues, environmental sources, clinical specimens and complex food matrices
- Superior assay sensitivity and specificity with ultrapure AccuStart II enzyme technology – maximum-yielding Taq DNA polymerase mutant controlled by stringent, multi-epitope antibody hot start
- Temperature stabilized master mix enables convenient setup at ambient room temperature

DESCRIPTION:

UltraPlex One-Step ToughMix is a ready-to-use, single-component SuperMix reagent for One-Step reverse transcription and real-time quantitative PCR (RT-qPCR) of RNA templates using probe-based detection methods. Advanced qScript XLT reverse transcriptase mutant possesses elevated temperature stability and improved template binding affinity increases sensitivity with large, complex RNA targets and delivers highly sensitive quantification with highly multiplexed RNA detection assays. ToughMix reagent technology ensures robust, reliable performance of highly-multiplexed (>4) RNA detection assays with a wide-range of inhibitory starting materials. This flexible formulation supports miniaturized reaction volumes (droplet PCR) with either fast or standard thermal cycling conditions.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
UltraPlex One-Step ToughMix- 100 R	95166-100	100 x 20 µl rxns
UltraPlex One-Step ToughMix - 500 R	95166-500	500 x 20 µl rxns
UltraPlex One-Step ToughMix - 1000 R	95166-01K	1000 x 20 µl rxns
UltraPlex One-Step ToughMix ROX - 100 R	95167-100	100 x 20 µl rxns
UltraPlex One-Step ToughMix ROX - 500 R	95167-500	500 x 20 µl rxns
UltraPlex One-Step ToughMix Low ROX - 100 R	95168-100	100 x 20 µl rxns
UltraPlex One-Step ToughMix Low ROX - 500 R	95168-500	500 x 20 µl rxns

Conventional PCR 3.1

AccuStart II PCR ToughMix

Robust, reliable PCR assay performance with challenging sample materials or impure templates

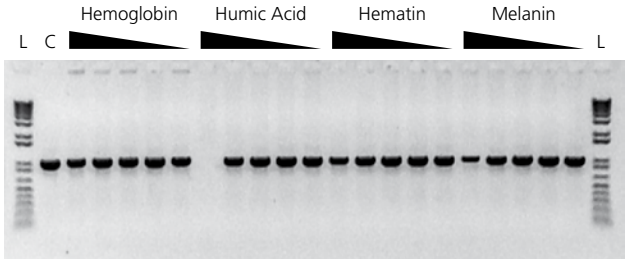
FEATURES AND BENEFITS:

- 1-tube SuperMix reagent minimizes pipetting, simplifies reaction assembly and improves accuracy
- ToughMix reagent technology neutralizes a broad spectrum of PCR inhibitors common in plant and animal tissues, environmental sources, clinical specimens and complex food matrices
- Superior assay sensitivity and specificity with ultrapure AccuStart II enzyme technology – maximum-yielding Taq DNA polymerase mutant controlled by stringent, multi-epitope antibody hot start
- Includes optional GelTrack dye to streamline gel electrophoresis workflows

DESCRIPTION:

AccuStart II PCR ToughMix is a 2x concentrated ready-to-use reaction cocktail for robust, general-purpose PCR amplification of DNA templates in the presence of PCR inhibitors. It contains all components, except primers and template. This reagent formulation contains an ultrapure, AccuStart II Taq DNA polymerase with stringent antibody hot start to ensure specific and efficient primer extension with convenient reaction assembly at ambient temperature. PCR products generally contain non-templated dA additions and can be cloned using vectors that have a single 3'-overhanging thymine residue on each end.

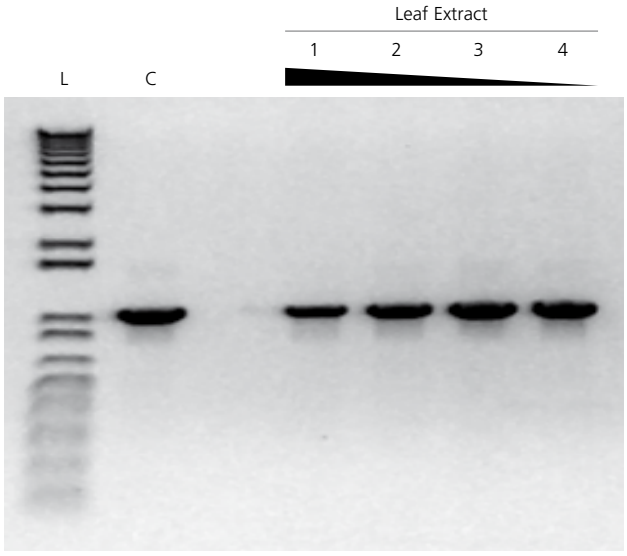
30 cycle PCR; 1 x 10^4 copies TcR DNA (1052 bp amplicon)



3.1 Inhibitor Resistance of AccuStart II PCR ToughMix. A 1-kb fragment from 1e4 copies of the Tetracyclin resistance gene was amplified in 20 µl reaction volumes according to the recommended protocol. Reactions were challenged with varying concentrations of different PCR inibitors as summarized below. Following a 3 min activation at 94°C; PCR was for 30 cycles of: 94°C, 15 s; 60°C, 20 s; 72°C, 1 min. 1/5th of each reaction was analyzed on a 01% agarose, 0.5x TBE gel conatining 0.25 mg/ml ethidium bromide.

Hemoglobin: 316 ng/µl, 100 ng/µl, 31.6 ng/µl, 10 ng/µl, 3.16 ng/µl
Humic Acid: 31.6 ng/µl, 10 ng/µl, 3.16 ng/µl, 1 ng/µl, 0.316 ng/µl
Hematin: 100 µM, 31.6 µM, 10 µM, 3.16 µM, 1 µM
Melanin: 10 ng/µl, 3.16 ng/µl, 1 ng/µl, 0.316 ng/µl, 0.1 ng/µl
C: control reactions without inhibitor; **L:** 1 Kb Plus DNA Ladder (Invitrogen)

30 cycle PCR; 1 x 10^4 copies TcR DNA (1052 bp amplicon)



3.2 **Inhibitor Resistance of AccuStart II PCR ToughMix: PCR in the presence of polyphenol spike.** Varying amounts of a polyphenol-rich plant extract (0.2, 0.06, 0.02, 0.006, or 0.002 µl) were added to 25 µl PCRs containing 10,000 copies of a control template. Amplification was carried out for 30 cycles of: 94°C, 15 s; 60°C, 20 s; 72°C, 1 min. 1/5th of each reaction was analyzed on a 01% agarose, 0.5x TBE gel containing 0.25 mg/ml ethidium bromide. As little as 0.002 µl of the crude plant lysate inhibited control reactions with a conventional PCR master mix (data not shown).

ORDER INFO

Product Name	Quantabio Catalog Number	Size
AccuStart II PCR ToughMix - 100 R	95142-100	100 x 25 µl rxns (1 x 1.25 ml)
AccuStart II PCR ToughMix - 800 R	95142-800	800 x 25 µl rxns (8 x 1.25 ml)
AccuStart II PCR ToughMix - 4000 R	95142-04K	4000 x 25 µl rxns (1 x 50 ml)

AccuStart Taq DNA Polymerase HiFi

Sensitive, precise amplification of large DNAs for high-fidelity downstream applications

FEATURES AND BENEFITS:

- Superior assay sensitivity and specificity with AccuStart enzyme technology – maximum-yielding Taq DNA polymerase mutant with stringent, multi-epitope antibody hot start
- Optimized high-fidelity PCR buffer with 3'-exonuclease (proofreading) activity supports robust amplification of large PCR products ≤20 kb in length

DESCRIPTION:

AccuStart Taq DNA Polymerase HiFi combines high-yielding mutant Taq DNA polymerase with 3'-exonuclease (proof-reading) polymerase and ultrapure, monoclonal antibody hot-start activation control. This reagent provides highly-sensitive and precise target amplification with convenient assembly at ambient temperature. Robust and reliable amplification of large, complex DNA targets up to 20 kb in length.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
AccuStart Taq DNA Polymerase HiFi - 250 U	95085-250	250 units (5 units/µl)
AccuStart Taq DNA Polymerase HiFi - 1000 U	95085-01K	1000 units (5 units/µl)
AccuStart Taq DNA Polymerase HiFi - 5000 U	95085-05K	5000 units (5 units/µl)

AccuStart II GelTrack PCR SuperMix

AccuStart II GelTrack PCR SuperMix is a 2x concentrated, ready-to-use reaction cocktail for routine PCR amplification of DNA fragments up to 4 kb

FEATURES AND BENEFITS:

- GelTrack Loading Dye pre-mixed
- High yield, high sensitivity
- Precise amplification – hot-start technology ensures specific and efficient primer extension
- Convenient reaction assembly at room temperature
- Preblended electrophoresis dyes to streamline gel electrophoresis workflows

DESCRIPTION:

AccuStart II GelTrack PCR SuperMix contains all components, except primers and template necessary for robust PCR. It simplifies reaction assembly, improves assay reproducibility, and reduces the risk of contamination. A key component is AccuStart II Taq DNA polymerase which contains monoclonal antibodies that bind to the polymerase and keep it inactive-prior to the initial PCR denaturation step. Upon heat activation (1 minute at 94°C), the antibodies denature irreversibly, releasing fully active, unmodified Taq DNA polymerase. This enables specific and efficient primer extension with the convenience of room temperature reaction assembly.

GelTrack Loading Dye is a mixture of blue and yellow electro-phoresis-tracking dyes that migrate at approximately 4kb and 50 bp, and comes pre-mixed with the PCR reagents.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
AccuStart II GelTrack PCR SuperMix - 100 R	95136-100	100 x 25 µl rxns (1 x 1.25 ml)
AccuStart II GelTrack PCR SuperMix - 500 R	95136-500	500 x 25 µl rxns (5 x 1.25 ml)
AccuStart II GelTrack PCR SuperMix - 4000 R	95136-04K	4000 x 25 µl rxns (1 x 50 ml)

AccuStart II PCR SuperMix

Robust, user-friendly 1-tube PCR SuperMix reagents for routine, general purpose PCR

FEATURES AND BENEFITS:

- 1-tube SuperMix reagent minimizes pipetting, simplifies reaction assembly and improves accuracy
- Sensitive, precise DNA amplification with ultrapure AccuStart II enzyme technology – maximum-yielding Taq DNA polymerase mutant controlled by stringent, multi-epitope antibody hot start

DESCRIPTION:

AccuStart II PCR SuperMix is a 2x concentrated, ready-to-use reaction cocktail for routine PCR amplification of DNA fragments up to 4 kb. 1-tube SuperMix reagent simplifies reaction assembly by minimizing pipetting steps and improving assay reproducibility. Ultrapure AccuStart II Taq DNA polymerase uses a stringent multi-epitope antibody hot-start that prevents non-specific primer extension prior to heat activation (1 minute at 94°C). The antibodies are irreversibly denatured, releasing a fully active, high-yielding Taq DNA polymerase mutant.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
AccuStart II PCR SuperMix - 100 R	95137-100	100 x 25 µl rxns (1 x 1.25 ml)
AccuStart II PCR SuperMix - 500 R	95137-500	500 x 25 µl rxns (5 x 1.25 ml)
AccuStart II PCR SuperMix - 4000 R	95137-04K	4000 x 25 µl rxns (1 x 50 ml)

AccuStart II Taq DNA Polymerase

AccuStart II Taq DNA Polymerase is a high purity, recombinant Taq DNA polymerase preparation with high avidity monoclonal antibodies that bind the polymerase and keep it inactive prior to the initial PCR denaturation step

FEATURES AND BENEFITS:

- Supports specific primer extension with AccuStart technology convenient room temperature reaction assembly

DESCRIPTION:

Upon heat activation (1 minute at 94°C), the antibodies denature irreversibly, releasing fully active, unmodified Taq DNA polymerase. The AccuStart II automatic hot-start enables specific and efficient primer extension in the PCR process with the added convenience of room temperature reaction assembly.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
AccuStart II Taq DNA Polymerase - 250 U	95141-250	250 U (5 U/µl)
AccuStart II Taq DNA Polymerase - 1000 U	95141-01K	1000 U (5 U/µl)
AccuStart II Taq DNA Polymerase - 5000 U	95141-05K	5000 U (5 U/µl)

5PRIME HotMaster Taq DNA Polymerase

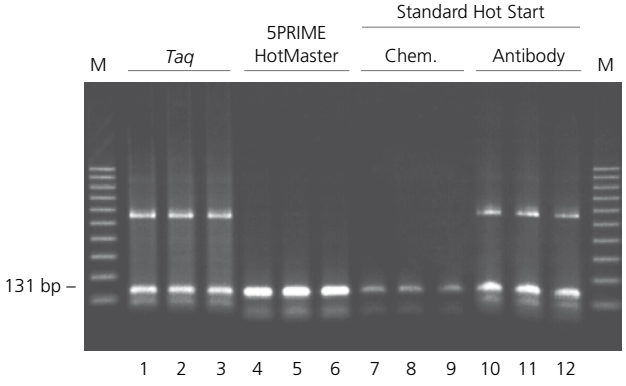
Innovative hot-start/cold-stop technology provides highly specific hot start PCR without activation step

FEATURES AND BENEFITS:

- Minimal optimization with self-adjusting magnesium buffer technology
- Novel HotMaster hot-start/cold-stop technology releases full activity at higher temperatures without activation step
- Convenient reaction set-up at ambient temperature

DESCRIPTION:

5PRIME HotMaster Taq DNA Polymerase uses an innovative thermostable Taq inhibitor that releases fully active at high temperatures ensuring precise primer annealing and extension at optimal temperatures and "cold-stop" after thermal cycling. Ideal for fast thermal cycling protocols.



3.3 Fast PCR Amplification of a 131 bp fragment of the human TNF gene with standard Taq, HotMaster Taq and conventional Hot Start enzymes. PCR protocol: 1 sec 95°C denaturation, 1 sec 55°C annealing, 5 sec 72°C extension. Initial denaturation was 2 min at 95°C prior to PCR or 10 min for the chemically modified enzyme respectively.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
HotMaster Taq DNA Polymerase - 100 U	2200300	100 U (5 U/µl)
HotMaster Taq DNA Polymerase - 1000 U	2200320	1000 U (5 U/µl)
HotMaster Taq DNA Polymerase - 5000 U	2200330	5000 U (5 U/µl)

5PRIME HotMasterMix

Highly specific hot-start PCR with minimal reaction setup

FEATURES AND BENEFITS:

- Optimal results – highly specific amplification
- Minimal handling – ready-to-use mastermix format
- Convenience – storage at 4°C eliminates freeze-thaw cycles

DESCRIPTION:

Highly specific amplification with minimal handling

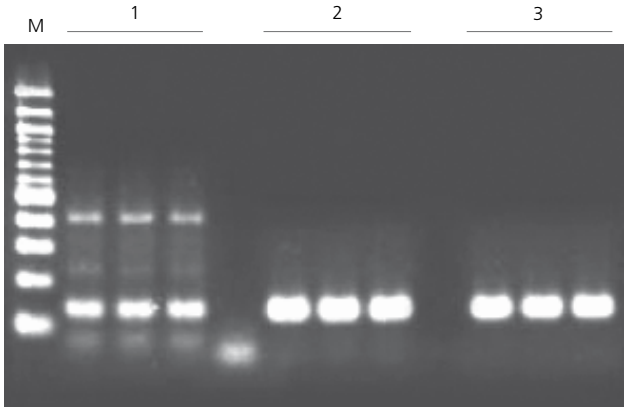
The 5PRIME HotMasterMix is a ready-to-use reagent mix that offers high reproducibility when processing large numbers of samples. The HotMaster Taq DNA Polymerase, an integral component of the master mix, is designed to reduce or eliminate any non-specific products that may result from mispriming during PCR.

The 5PRIME HotMasterMix (2.5x) is a ready-to-use PCR mix. Only primers and template need to be added to the 2.5x concentrate,thus decreasing the number of time-consuming pipetting steps. This format not only reduces the likelihood of errors and the risk of contamination, but it also increases precision and sample throughput.

The 5PRIME HotMasterMix also contains the self-adjusting Mg^{2+} buffer technology. This formulation adjusts the Mg^{2+} concentration automatically, eliminating the need for optimizing this critical component. The MasterMix does not need to be stored frozen, eliminating the time-consuming thawing process and the resulting reduction in performance.

The 5PRIME HotMasterMix is a 2.5-fold concentrate, resultingin the following final concentrations for a 50 µl PCR reaction: 1 U Taq DNA Polymerase, 45 mM Cl, 2.5 mM Mg^{2+}, 200 µM of each dNTP.

Comparison of 5PRIME Taq Enzymes



3.4 Amplification of a 131 bp TNF fragment using different 5PRIME Taq enzymes.

M 100 bp Marker
1 Standard Taq
2 HotMaster Taq
3 HotMasterMix

ORDER INFO

Product Name	Quantabio Catalog Number	Size
5PRIME HotMasterMix - 100 R	2200400	100 x 50 µl rxns (2 x 1 ml)
5PRIME HotMasterMix - 1000 R	2200410	1000 x 50 µl rxns (20 x 1 ml)

10 mM dNTP Mix

DESCRIPTION:

The 10 mM dNTP Mix is a solution of high purity deoxyribonucleoside triphosphates that has been functionally qualified for real-time quantitative PCR (qPCR). It is suitable for use in conventional end-point PCR, real-time qPCR, first-strand cDNA synthesis, as well as other applications that require dNTP as substrate.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
10 mM dNTP Mix - 200 µl	95062-200	200 µl
10 mM dNTP Mix - 1000 µl	95062-01K	1000 µl

3.2 Real-Time Quantitative PCR

Multiplexed Pre-Amplification

PerfeCTa PreAmp 5x SuperMix

**Unbiased pre-amplification of up to 100 DNA targets from as little as 100 pg total cDNA.
Compatible with either probe-based or dye-based qPCR detection chemistries**

FEATURES AND BENEFITS:

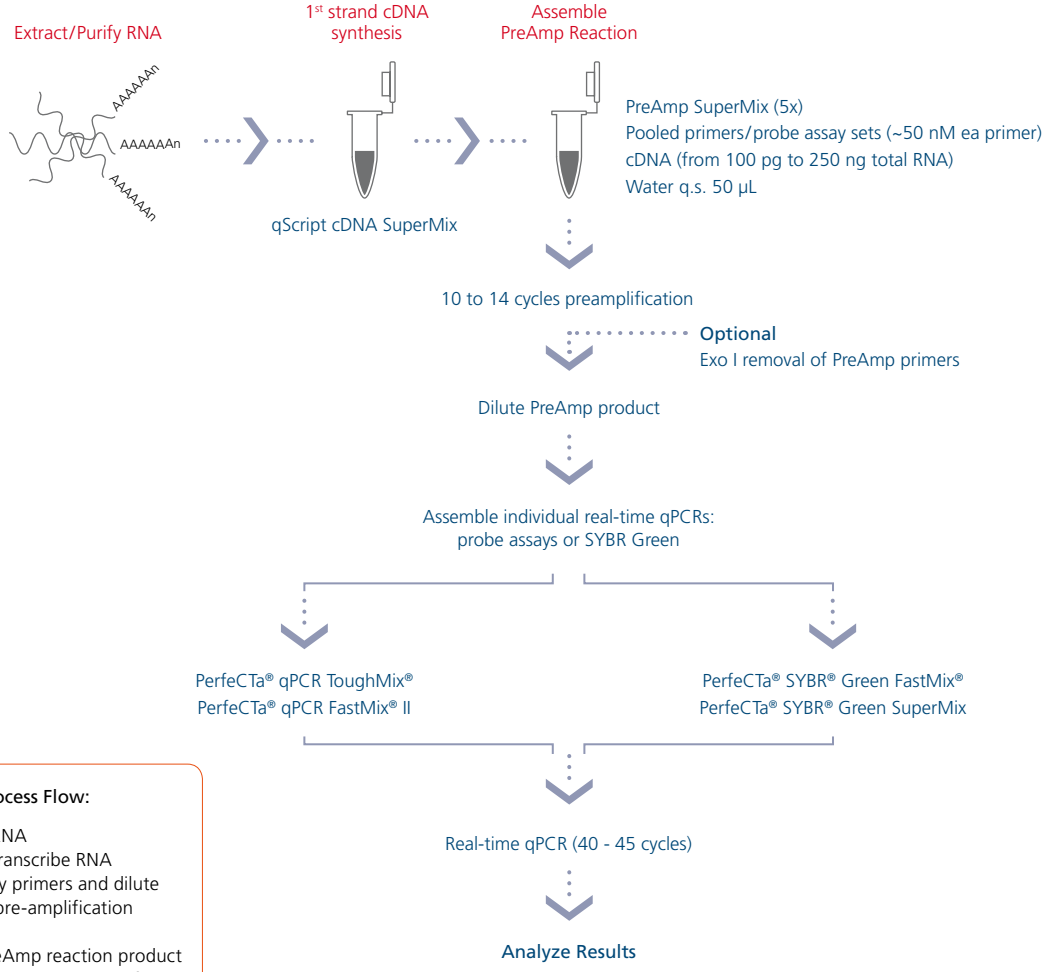
- Unbiased, linear pre-amplification of up to 100 DNA targets
- 5x concentrated SuperMix maximizes sample input volume with dilute cDNA templates
- Superior assay sensitivity and specificity with ultrapure AccuStart II enzyme technology – maximum-yielding Taq DNA polymerase mutant controlled by stringent, multi-epitope antibody hot-start
- User-friendly inert AccuVue plate loading dye provides visual confirmation of reagent addition
- Supports efficient vortex mixing

DESCRIPTION:

PerfeCTa PreAmp SuperMix is a 5x concentrated, ready-to-use reaction cocktail for unbiased, selected enrichment of target sequences from limiting amounts of starting material for downstream gene expression profiling or targeted re-sequencing. It contains all the necessary components, except primers and templates. The 5x concentrated SuperMix allows addition of higher template volumes when working with low concentration samples, and/or reduced reaction volumes. Inclusion of inert light blue tracer dye helps visualize small reaction volumes and ensure accurate pipetting. PerfeCTa PreAmp SuperMix delivers unbiased pre-amplification of up to 100 target sequences from as little as 100 pg of total cDNA. It is compatible with both TaqMan 5'-nuclease probes or ds-DNA binding dye (i.e. SYBR Green I) qPCR detection chemistries.

A key component of PerfeCTa PreAmp SuperMix is an ultra pure, highly processive thermostable DNA polymerase that is combined with high avidity monoclonal antibodies. This proprietary polymerase mix is resistant to PCR inhibitors and provides an extremely stringent automatic hot-start allowing reaction assembly, and temporary storage, at room temperature prior to pre-amplification.

PreAmp Process Flow



PreAmp Process Flow:

1. Prepare RNA
2. Reverse transcribe RNA
3. Pool assay primers and dilute
4. Perform pre-amplification reaction
5. Dilute PreAmp reaction product
6. Perform individual qPCRs for each pre-amplified gene of interest (GOI).

ORDER INFO

Product Name	Quantabio Catalog Number	Size
PerfeCTa PreAmp 5x SuperMix - 5 R	95146-005	5 x 50 µl rxns
PerfeCTa PreAmp 5x SuperMix - 40 R	95146-040	40 x 50 µl rxns

SYBR Green Detection

PerfeCTa SYBR Green SuperMix/FastMix

Sensitive and precise DNA amplification with DNA-intercalating dye based detection chemistry

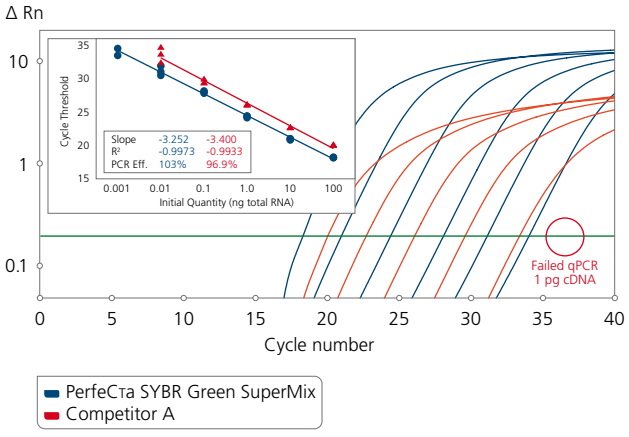
FEATURES AND BENEFITS:

- 2x concentrated reagents minimize pipetting steps, simplify reaction assembly and improve accuracy
- Superior assay sensitivity and specificity with ultrapure AccuStart enzyme technology – maximum-yielding Taq DNA polymerase mutant controlled by stringent, multi-epitope antibody hot start
- Supports efficient vortex mixing with proprietary anti-foaming formulation
- FastMix formulation supports both fast and standard thermal cycling conditions
- SuperMix version provides maximum dye concentration for robust optical signal with small amplicons (i.e. microRNA-templated cDNA)

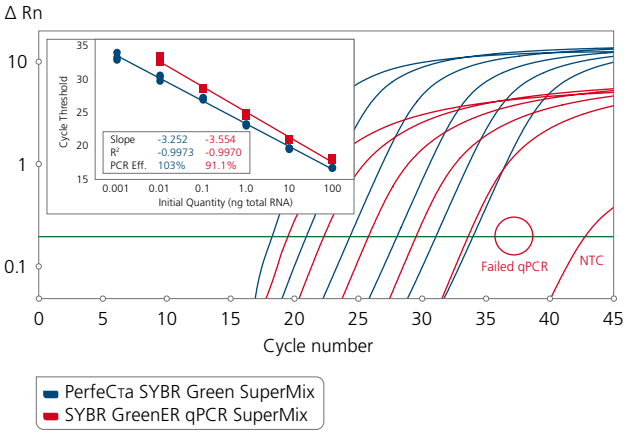
DESCRIPTION:

Specific target amplification is essential for precise target quantification with SYBR Green I technology since this dye binds to all dsDNA generated during amplification. PerfeCTa SYBR Green SuperMix and FastMix ensure specific primer extension products with ultra-pure AccuStart hot start technology and proprietary formulation that reduces potential for primer-dimer and other non-specific artifacts.

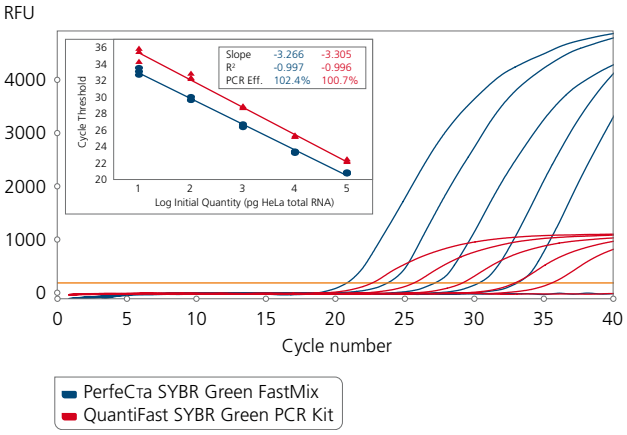
Single-tube reagents are 2x concentrated ready-to-use reaction cocktails containing all necessary components, except primers and DNA template for quantitative PCR. Proprietary formulation stabilizes SYBR Green I dye to deliver maximum efficiency, sensitivity, and robust fluorescent signal.



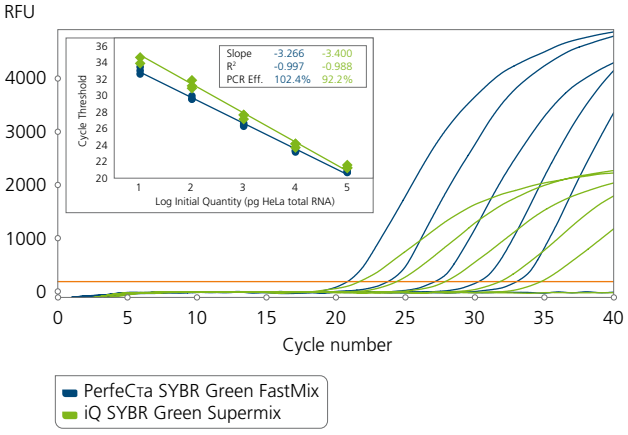
3.7 PKCA target amplified from log-fold serial dilutions of qScript synthesized cDNA from HeLa cell total RNA (100 ng – 1 pg) with either PerfeCTa SYBR Green SuperMix or Power SYBR Green PCR Master Mix.



3.8 PKCA target amplified from log-fold serial dilutions of qScript synthesized cDNA from HeLa cell total RNA (100 ng – 1 pg) with either PerFeCTa SYBR Green SuperMix or SYBR GreenER™ qPCR SuperMix.



3.9 ADAR target amplified from log-fold serial dilutions of qScript synthesized cDNA from HeLa cell total RNA (100 ng – 10 pg) with either PerfeCTa SYBR Green FastMix or QuantiFast SYBR Green PCR Kit.



3.10 ADAR target amplified from log-fold serial dilutions of qScript synthesized cDNA from HeLa cell total RNA (100 ng – 10 pg) with either PerFeCTa SYBR Green FastMix or iQ™ SYBR Green Supermix.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
PerfeCta SYBR Green FastMix for iQ - 250 R	95071-250	2 x 1.25 ml
PerfeCta SYBR Green FastMix for iQ - 1250 R	95071-012	10 x 1.25 ml
PerfeCta SYBR Green FastMix for iQ - 5000 R	95071-05K	1 x 50 ml
PerfeCta SYBR Green FastMix - 250 R	95072-250	2 x 1.25 ml
PerfeCta SYBR Green FastMix - 1250 R	95072-012	10 x 1.25 ml
PerfeCta SYBR Green FastMix - 5000 R	95072-05K	1 x 50 ml
PerfeCta SYBR Green FastMix, ROX - 250 R	95073-250	2 x 1.25 ml
PerfeCta SYBR Green FastMix, ROX - 1250 R	95073-012	10 x 1.25 ml
PerfeCta SYBR Green FastMix, ROX - 5000 R	95073-05K	1 x 50 ml
PerfeCta SYBR Green FastMix, Low ROX - 250 R	95074-250	2 x 1.25 ml
PerfeCta SYBR Green FastMix, Low ROX - 1250 R	95074-012	10 x 1.25 ml
PerfeCta SYBR Green FastMix, Low ROX - 5000 R	95074-05K	1 x 50 ml
PerfeCta SYBR Green SuperMix for iQ - 250 R	95053-100	2 x 1.25 ml
PerfeCta SYBR Green SuperMix for iQ - 1250 R	95053-500	10 x 1.25 ml
PerfeCta SYBR Green SuperMix for iQ - 5000 R	95053-02K	1 x 50 ml
PerfeCta SYBR Green SuperMix - 250 R	95054-100	2 x 1.25 ml
PerfeCta SYBR Green SuperMix - 1250 R	95054-500	10 x 1.25 ml
PerfeCta SYBR Green SuperMix - 5000 R	95054-02K	1 x 50 ml
PerfeCta SYBR Green SuperMix, ROX - 250 R	95055-100	2 x 1.25 ml
PerfeCta SYBR Green SuperMix, ROX - 1250 R	95055-500	10 x 1.25 ml
PerfeCta SYBR Green SuperMix, ROX - 5000 R	95055-02K	1 x 50 ml
PerfeCta SYBR Green SuperMix, Low ROX - 250 R	95056-100	2 x 1.25 ml
PerfeCta SYBR Green SuperMix, Low ROX - 1250 R	95056-500	10 x 1.25 ml
PerfeCta SYBR Green SuperMix, Low ROX - 5000 R	95056-02K	1 x 50 ml

Probe-based Detection

PerfeCTa qPCR ToughMix

Robust, inhibitor-resistant probed-based qPCR

FEATURES AND BENEFITS:

- 2x concentrated reagents minimize pipetting steps, simplify reaction assembly and improve accuracy
- ToughMix reagent technology neutralizes a broad spectrum of PCR inhibitors common in plant and animal tissues, environmental sources, clinical specimens and complex food matrices
- Superior assay sensitivity and specificity with ultrapure AccuStart II enzyme technology – maximum-yielding Taq DNA polymerase mutant controlled by stringent, multi-epitope antibody hot start
- Easy-to-use 2x concentrated master mixes with AccuVue plate loading dye and optimized passive reference dye for simplified reaction setup
- Supports efficient vortex mixing with proprietary anti-foaming technology

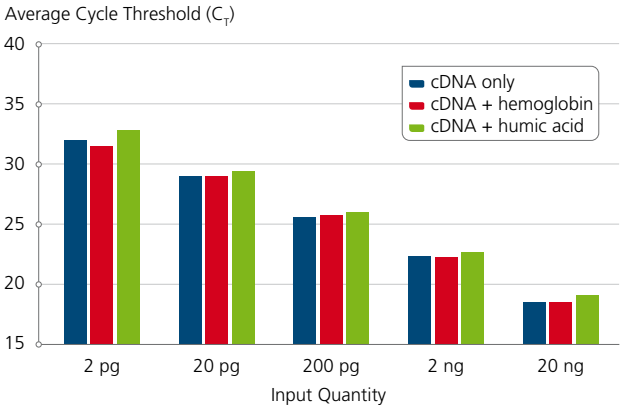
DESCRIPTION:

PerfeCTa qPCR ToughMix is a 2x concentrated qPCR SuperMix ready-to-use reaction cocktail for PCR amplification of DNA templates that overcomes a broad spectrum of PCR inhibitors often encountered in environmental specimens, plant tissues or animal tissues. This proprietary polymerase mix provides maximum sensitivity and PCR efficiency with all dual-label (hydrolysis) probe-based detection chemistries and stringent hot-start activation control allowing reaction assembly pre-run storage at ambient room temperature prior to thermal cycling. Inert AccuVue plate loading dye is compatible with either white or clear PCR plates and helps to minimize pipette error and provides visual confirmation of reagent addition. UNG containing versions are blended with Uracil N-glycosylase to eliminate potential post-PCR carryover contamination associated with routine testing workflows.

Inhibitor	Common sources	Reagent performance	
		Competitor	PerfeCTa ToughMix
Polyphenols	Plant extracts	–	✓
Humic acids	Soil Plant tissues	–	✓
Hematin	Dried bloods Blood spots	–	✓
Hemoglobin	Blood	✓	✓
Polysaccharides	Feces Plant tissues	–	✓
Melanin	Hair Skin	–	✓

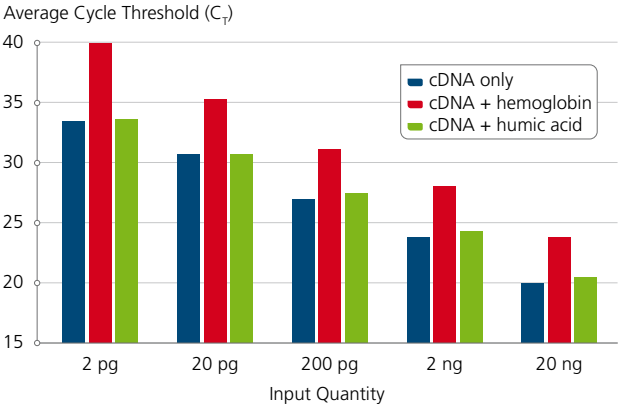
Effect of PCR Inhibitors on qPCR of MYC cDNA

PerfeCTa qPCR ToughMix



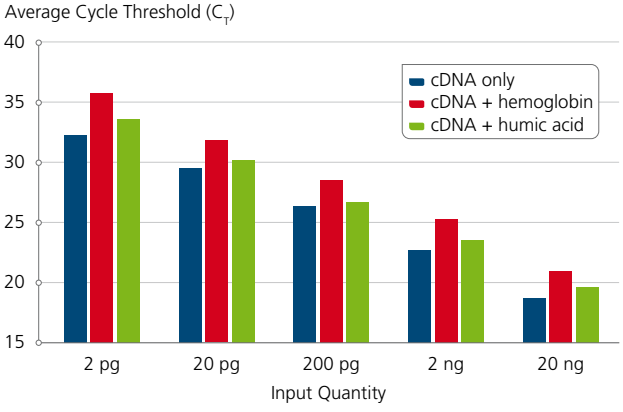
Slope	-3.365	-3.267	-3.420
R^2	-0.9989	-0.9970	-0.9988
Int.	27.57	27.42	28.08
PCR Eff.	98.2%	102.4%	96.1%

Path-ID™ qPCR Master Mix



Slope	-3.376	-3.935	-3.258
R^2	-0.9968	-0.9953	-0.9989
Int.	27.07	33.93	29.32
PCR Eff.	97.7%	79.5%	102.7%

TaqMan Environmental Master Mix 2.0



Slope	-3.409	-3.616	-3.480
R^2	-0.9968	-0.9965	-0.9989
Int.	28.14	30.77	28.92
PCR Eff.	96.5%	89.0%	93.8%

3.11 **Serial dilutions of qScript cDNA.** cDNA alone, cDNA + 1 µg hemoglobin, cDNA + 10 ng/µl humic acid (100 ng/rxn). 10 µl reactions; Roche LC480; 384-well optimal cycling for TaqMan reagents: 95°C, 10 min; followed by 45 cycles of 95°C, 15 s; 60°C, 60 s. 0.5x MYC (FAM-MGB) TaqMan Gene Expression Assay.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
PerfeCta qPCR ToughMix - 250 R	95112-250	250 x 20 µl rxns (2 x 1.25 ml)
PerfeCta qPCR ToughMix - 1250 R	95112-012	1250 x 20 µl rxns (10 x 1.25 ml)
PerfeCta qPCR ToughMix - 5000 R	95112-05K	5000 x 20 µl rxns (1 x 50 ml)
PerfeCta qPCR ToughMix, ROX - 250 R	95113-250	250 x 20 µl rxns (2 x 1.25 ml)
PerfeCta qPCR ToughMix, ROX - 1250 R	95113-012	1250 x 20 µl rxns (10 x 1.25 ml)
PerfeCta qPCR ToughMix, ROX - 5000 R	95113-05K	5000 x 20 µl rxns (1 x 50 ml)
PerfeCta qPCR ToughMix, Low ROX - 250 R	95114-250	250 x 20 µl rxns (2 x 1.25 ml)
PerfeCta qPCR ToughMix, Low ROX - 1250 R	95114-012	1250 x 20 µl rxns (10 x 1.25 ml)
PerfeCta qPCR ToughMix, Low ROX - 5000 R	95114-05K	5000 x 20 µl rxns (1 x 50 ml)
PerfeCta qPCR ToughMix, UNG - 250 R	95138-250	250 x 20 µl rxns (2 x 1.25 ml)
PerfeCta qPCR ToughMix, UNG - 1250 R	95138-012	1250 x 20 µl rxns (10 x 1.25 ml)
PerfeCta qPCR ToughMix, UNG - 5000 R	95138-05K	5000 x 20 µl rxns (1 x 50 ml)
PerfeCta qPCR ToughMix, UNG, ROX - 250 R	95139-250	250 x 20 µl rxns (2 x 1.25 ml)
PerfeCta qPCR ToughMix, UNG, ROX - 1250 R	95139-012	1250 x 20 µl rxns (10 x 1.25 ml)
PerfeCta qPCR ToughMix, UNG, ROX - 5000 R	95139-05K	5000 x 20 µl rxns (1 x 50 ml)
PerfeCta qPCR ToughMix, UNG, Low ROX - 250 R	95140-250	250 x 20 µl rxns (2 x 1.25 ml)
PerfeCta qPCR ToughMix, UNG, Low ROX - 1250 R	95140-012	1250 x 20 µl rxns (10 x 1.25 ml)
PerfeCta qPCR ToughMix, UNG, Low ROX - 5000 R	95140-05K	5000 x 20 µl rxns (1 x 50 ml)

PerfeCta FastMix II

Convenient, 1-tube reagent solution supports robust and reliable probe-based DNA detection with fast or standard thermal cycling conditions

FEATURES AND BENEFITS:

- 2x concentrated reagents minimize pipetting steps, simplify reaction assembly and improve accuracy
- Superior assay sensitivity and specificity with ultrapure AccuStart II enzyme technology – maximum-yielding Taq DNA polymerase mutant controlled by stringent, multi-epitope antibody hot-start
- Inert AccuVue plate loading dye simplifies reaction setup and provides instant visual cue of reagent addition and mixing.
- Supports efficient vortex mixing with proprietary anti-foaming technology
- Flexible – optimized for both fast and standard thermal cycling conditions

DESCRIPTION:

PerfeCta FastMix II is an advanced 2x concentrated qPCR SuperMix reagent for both fast and conventional PCR cycling protocols or instruments. It is a versatile and robust solution that provides the superior sensitivity and high PCR efficiency and is compatible with all dual-label (hydrolysis) probe chemistries. The kit is provided as a 2x concentrated ready-to-use reaction cocktail that contains all required reaction components, except primers, probe(s), and DNA template. Inert AccuVue plate loading dye helps to minimize pipette error and provides visual confirmation of thorough mixing.

A key component of the kit is an ultrapure, highly-processive thermostable DNA polymerase that is free of detectable *E. coli* DNA. PerfeCta FastMix II is ideal for demanding qPCR applications such as bacterial pathogen detection where residual host DNA in typical recombinant enzyme preparations can limit assay sensitivity and obscure detection of low copy samples.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
PerfeCta qPCR FastMix II - 250 R	95118-250	250 x 20 µl rxns (2 x 1.25 ml)
PerfeCta qPCR FastMix II - 1250 R	95118-012	1250 x 20 µl rxns (10 x 1.25 ml)
PerfeCta qPCR FastMix II - 5000 R	95118-05K	5000 x 20 µl rxns (1 x 50 ml)
PerfeCta qPCR FastMix II, ROX - 250 R	95119-250	250 x 20 µl rxns (2 x 1.25 ml)
PerfeCta qPCR FastMix II, ROX - 1250 R	95119-012	1250 x 20 µl rxns (10 x 1.25 ml)
PerfeCta qPCR FastMix II, ROX - 5000 R	95119-05K	5000 x 20 µl rxns (1 x 50 ml)
PerfeCta qPCR FastMix II, Low ROX - 250 R	95120-250	250 x 20 µl rxns (2 x 1.25 ml)
PerfeCta qPCR FastMix II, Low ROX - 1250 R	95120-012	1250 x 20 µl rxns (10 x 1.25 ml)
PerfeCta qPCR FastMix II, Low ROX - 5000 R	95120-05K	5000 x 20 µl rxns (1 x 50 ml)

PerfeCta MultiPlex qPCR ToughMix

Advanced 1-tube SuperMix optimized to support highly multiplexed DNA amplifications in miniaturized reaction volumes and withstand a broad spectrum of PCR inhibitors

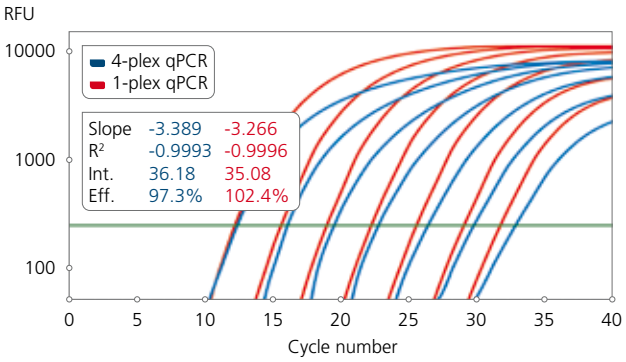
FEATURES AND BENEFITS:

- 1-tube SuperMix minimizes pipetting steps, simplifying reaction assembly and improving accuracy
- 5x concentrated reagent provides more flexibility with dilute DNA samples sensitivity
- ToughMix reagent technology neutralizes a broad spectrum of PCR inhibitors common in plant and animal tissues, environmental sources, clinical specimens and complex food matrices
- Superior assay sensitivity and specificity with ultrapure AccuStart II enzyme technology – maximum-yielding Taq DNA polymerase mutant controlled by stringent, multi-epitope antibody hotstart
- Supports efficient vortex mixing with proprietary anti-foaming additives

DESCRIPTION:

Suppression of low copy amplicons by high copy reference targets during multiplex co-amplification skews the apparent representation and quantification of low copy target sequences. PerfeCta MultiPlex qPCR ToughMix transcends these limitations by enabling sensitive, broad linear dynamic detection range with co-amplification of four abundant (10^6) targets. PerfeCta MultiPlex qPCR ToughMix enables multiplex qPCR assay performance comparable to single-plex qPCR probe assays without the need to rigorously titrate primer concentration.

PerfeCta MultiPlex qPCR ToughMix is a 5x concentrated, ready-to-use reaction cocktail for real-time quantitative PCR (qPCR) with ToughMix reagent technology that neutralizes a broad spectrum of PCR inhibitors that compromise assay performance with crude extracts, clinical specimens, plant, soil environmental or complex food matrices. A key component of the kit is an ultrapure, highly-processive thermostable DNA polymerase that is free of detectable *E. coli* DNA. PerfeCta FastMix II is ideal for demanding qPCR applications such as bacterial pathogen detection where residual host DNA in typical recombinant enzyme preparations can limit assay sensitivity and obscure detection of low copy samples.



3.12 **High efficiency, high sensitivity multiplex qPCR results with PerfeCta MultiPlex qPCR ToughMix**. Log-fold serial dilutions (10 to 1 E7 copies) of a plasmid containing the GAPDH gene, as well as no template controls, were amplified with PerfeCta MultiPlex qPCR ToughMix as either a single-plex qPCR, or a 4-target multiplexed qPCR that contained 1 E8 copies of 3 additional plasmid DNAs (ACTB, IL1beta, and TUBA). Quadruplicate reactions for each input quantity were carried out in 25 µl volumes with 300 nM each primer and 150 nM each probe. Dual-labeled probes with non-fluorescent quenchers were from Biosearch Technologies. GAPDH was detected using a FAM-BHQ1 probe. ACTB; CAL Fluor Orange 560-BHQ1; IL1beta: CAL Fluor Red 610-BHQ2; TUBA: Quasar 670 – BHQ3. Single-plex qPCRs only contained the GAPDH primers and probe. Cycling was performed on a Bio-Rad CFX with the following protocol. 95°C, 2 min; followed by 40 cycles of 95°C, 10 s; 58°C, 90 s. RFU data were exported to Excel, averaged for each replicate reaction series, and plotted.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
PerfeCta MultiPlex qPCR ToughMix - 250 R	95147-250	250 x 25 µl rxns (1 x 1.25 ml)
PerfeCta MultiPlex qPCR ToughMix - 1000 R	95147-01K	1000 x 25 µl rxns (4 x 1.25 ml)
PerfeCta MultiPlex qPCR ToughMix - 5000 R	95147-05K	5000 x 25 µl rxns (1 x 25 ml)
PerfeCta MultiPlex qPCR ToughMix, ROX - 250 R	95148-250	250 x 25 µl rxns (1 x 1.25 ml)
PerfeCta MultiPlex qPCR ToughMix, ROX - 1000 R	95148-01K	1000 x 25 µl rxns (4 x 1.25 ml)
PerfeCta MultiPlex qPCR ToughMix, ROX - 5000 R	95148-05K	5000 x 25 µl rxns (1 x 25 ml)
PerfeCta MultiPlex qPCR ToughMix, Low ROX - 250 R	95149-250	250 x 25 µl rxns (1 x 1.25 ml)
PerfeCta MultiPlex qPCR ToughMix, Low ROX - 1000 R	95149-01K	1000 x 25 µl rxns (4 x 1.25 ml)
PerfeCta MultiPlex qPCR ToughMix, Low ROX - 5000 R	95149-05K	5000 x 25 µl rxns (1 x 25 ml)

PerfeCTa MultiPlex qPCR SuperMix

Sensitive and robust multiplex qPCR assay performance with user-friendly 1-tube reagents containing pre-blended passive reference dye

FEATURES AND BENEFITS:

- Robust assay performance for highly-multiplexed DNA quantification assays
- 2x concentrated reagents minimize pipetting steps, simplify reaction assembly and improve accuracy.
- Superior assay sensitivity and specificity with ultrapure AccuStart II enzyme technology – maximum-yielding Taq DNA polymerase mutant controlled by stringent, multi-epitope antibody hot start
- Broad linear detection range with highly-multiplexed qPCR assays
- Supports efficient vortex mixing with proprietary anti-foaming additives

DESCRIPTION:

Suppression of low abundance targets by high abundance reference targets during co-amplification is a common problem in multiplex PCR in which individual assay sensitivity can be significantly compromised. PerfeCTa Multiplex qPCR SuperMix delivers assay performance with exceptionally road, linear detection and limit-of-detection (LOD) sensitivity.

Multiplexed qPCR performance comparable to single-plex assay performance without the need for rigorous titration of individual primer assays is achievable with this reagent.

PerfeCTa Multiplex qPCR SuperMix is a 2x concentrated, ready-to-use reaction cocktail that contains all the necessary components except: primers, probe(s), and DNA template for highly-multiplexed, real-time quantitative PCR. This reagent formulation pushes the boundary of multiplex qPCR by enabling unbiased amplification of up to 5 targets in a single amplification reaction. A key component of the kit is an ultrapure, highly-processive thermostable DNA polymerase that is free of detectable *E. coli* DNA. PerfeCTa FastMix II is ideal for demanding qPCR applications such as bacterial pathogen detection where residual host DNA in typical recombinant enzyme preparations can limit assay sensitivity and obscure detection of low copy samples.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
PerfeCTa MultiPlex qPCR SuperMix - 50 R*	95063-050	50 x 50 µl rxns (1 x 1.25 ml)
PerfeCTa MultiPlex qPCR SuperMix - 200 R*	95063-200	200 x 50 µl rxns (4 x 1.25 ml)
PerfeCTa MultiPlex qPCR SuperMix - 1000 R*	95063-01K	1000 x 50 µl rxns (1 x 25 ml)
PerfeCTa MultiPlex qPCR SuperMix, Low ROX - 50 R	95108-050	50 x 50 µl rxns (1 x 1.25 ml)
PerfeCTa MultiPlex qPCR SuperMix, Low ROX - 200 R	95108-200	200 x 50 µl rxns (4 x 1.25 ml)
PerfeCTa MultiPlex qPCR SuperMix, Low ROX - 1000 R	95108-01K	1000 x 50 µl rxns (1 x 25 ml)

* Contains seperate tube of 50x ROX and 50x Low Rox

repliQa HiFi Assembly Mix

Seamless assembly of multiple DNA fragments for high efficiency cloning

FEATURES & BENEFITS:

- Formulated to increase number of transformants
- Assemble up to 6 fragment inserts without the need for restriction enzymes
- Flexibility in design with 10x master mix enabling assembly of low concentration DNA samples
- Eliminates dilution step resulting in easier workflow and 1 hr assembly
- Includes DpnI to reduce background when using plasmid templates for PCR

DESCRIPTION:

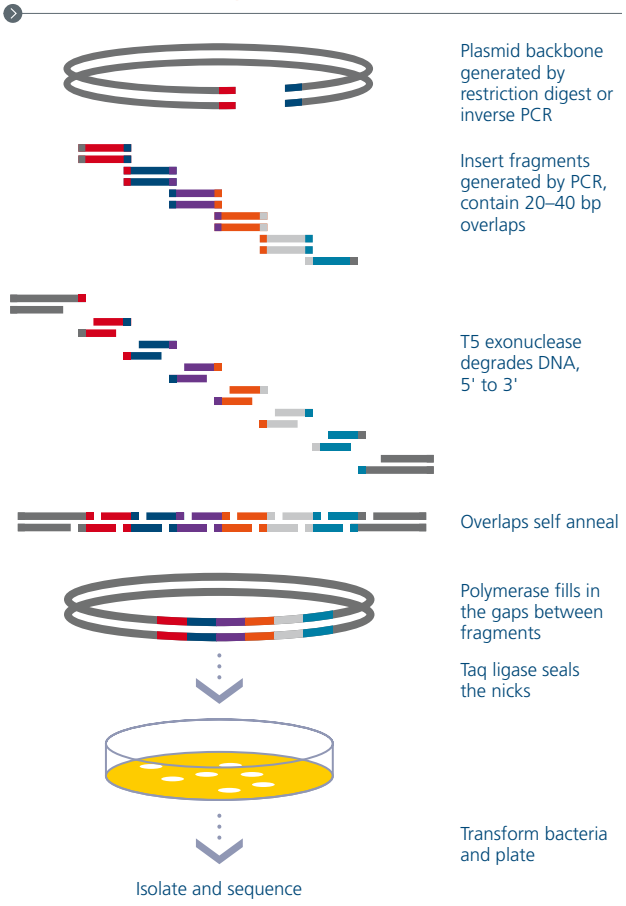
The repliQa HiFi Assembly Mix simplifies the construction of recombinant DNA through seamless assembly of multiple DNA fragments in a single, isothermal reaction.

Similar in principle to the Gibson Assembly® Method, the high efficiency repliQa HiFi Assembly Mix is ideal for a range of genetic engineering applications including:

- Routine molecular cloning
- Site-directed mutagenesis
- Synthetic biology
- Construction of libraries for directed evolution studies

The concentrated (10x), two component format allows flexibility in design of assembly reactions and compatibility with less concentrated DNA samples. The repliQa Mix has been optimized for use with a total input quantity of DNA fragments in the range of 0.03 to 0.5 pmols. Assembly of up to six DNA fragments is recommended, though the repliQa Mix has been used successfully for more complex assemblies. The mix supports assembly of multiple DNA fragments in a single 1-hr reaction.

repliQa Procedure



Speed up your workflow and increase transformation efficiency

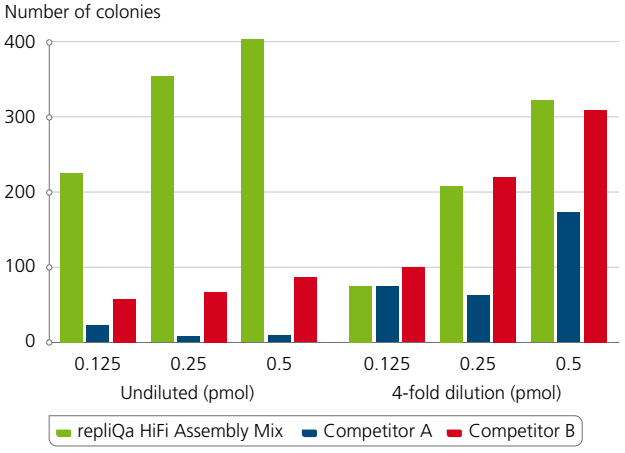
repliQa HiFi Assembly Mix increases transformation efficiency without the need for diluting or purifying the assembly reaction prior to transformation of competent cells, resulting in less hands-on time and faster workflows.

Low DNA amounts can be used and will efficiently generate high amounts of transformats.

Assemble larger number of fragments

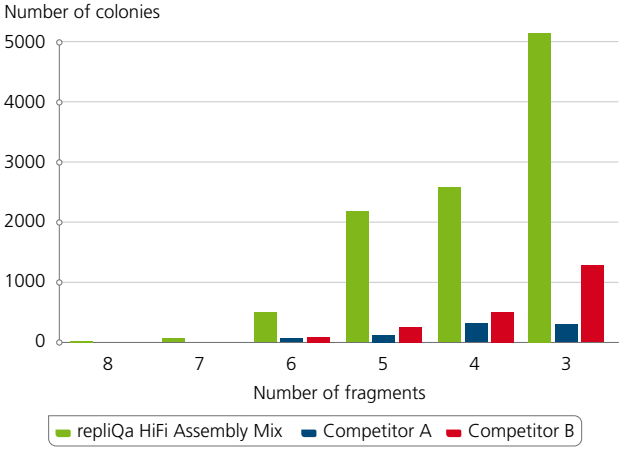
repliQa HiFi Assembly Mix allows for large inserts to be cloned at a very high efficiency resulting in significantly more positive clones eliminating the need to repeat experiments due to erroneous or insufficient clones.

Dilution of the assembly reaction not required – high transformation efficiency, less hands-on time



3.5 Three DNA fragments containing 23 bp overlaps were generated by PCR, DpnI treated and purified. The three fragments, 4.2 kb, 3.1 kb, and 400 bp in size, were combined in a 1:1.4:5 molar ratio. Total DNA quantities used are indicated (x-axis) and reacted at 50 °C for 60 minutes according to the protocol. One microliter of the undiluted assembled products or one microliter of a 4-fold dilution of the assembled products was used to transform 30 µl of chemically competent cells.

High transformation efficiency results in greater number of fragments



3.6 PCR fragments containing 30 bp overlaps were DpnI treated, purified, and assembled according to the protocol. Reactions contained the indicated number of DNA fragments (0.1 pmol each) and were incubated at 50 °C for 60 minutes. 1 µl of the reactions were used to transform 30 µl of chemically competent cells.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
repliQa HiFi Assembly Mix, 10 rxns	95190-010	10 rxns
repliQa HiFi Assembly Mix, 50 rxns	95190-050	50 rxns

qPCR cycler

A faster, smaller, better way to qPCR.

FEATURES AND BENEFITS:

- **Ultra-Fast Data Acquisition** – 35 cycles in 25 minutes*
- **Unrivaled Performance** – Detect 2-fold expression level differences
- **Portable & Compact** – 4.5 lbs - transport without ever calibrating
- **Scalable & Wireless** – Connect up to 10 instruments (48 samples/ instrument)
- **Magnetic Induction** – Eliminate variability vs block-based cyclers

DESCRIPTION:

Q uses a patented magnetic induction technology to rapidly heat samples coupled with fan forced air for cooling to acquire data in as little as 25 minutes. Available in 2 or 4 channel models, the robust optical system acquires all channels simultaneously and allows for running the fastest multiplexed assays.

Q's miniature speaker-size and 4.5 pound weight make it the most portable and versatile qPCR cycler on the market without ever needing to calibrate. Q also provides scalability as each instrument can process up to 48 samples per run and up to 10 Q's can be connected to a single computer wirelessly via bluetooth enabling up to 480 samples to be processed simultaneously.

A key difference is that Q incorporates a unique spinning aluminum rotor providing superior temperature uniformity of ± 0.05°C versus traditional block-based real time cyclers which rely on multiple peltier heating blocks that can create edge effects resulting in sample variation. Not only does the data give you superior reproducibility, repeatability but enables detection of 2-fold gene expression level differences as well as identification of difficult class IV SNP's requiring melt temperature resolutions of 0.1°C.

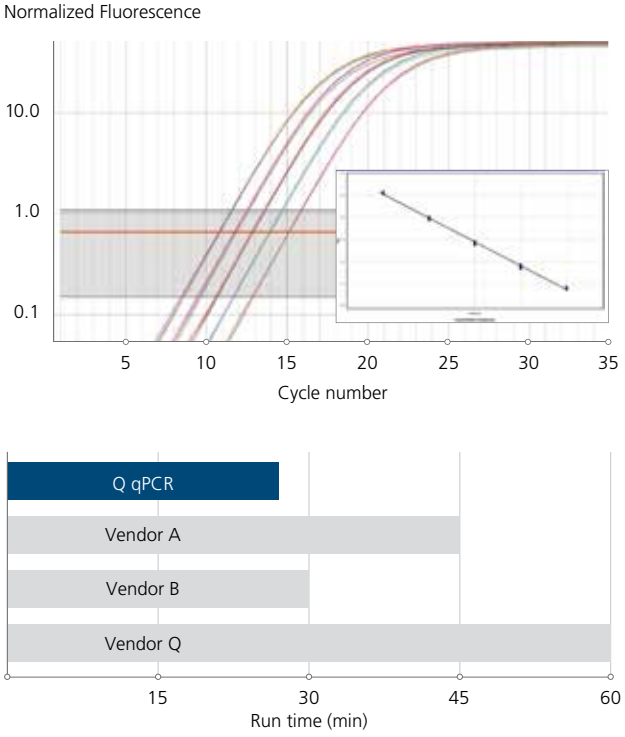
3.7 Q qPCR cycler.

* 25 minute cycle times obtained with fast cycling master mixes and short amplicon assay designs targeting cDNA.

Ultra-Fast Data Acquisition

Generate high quality data, fast!

- Q's speed is the fastest in the industry
- Don't sacrifice on the performance quality of your qPCR
- Completing runs in as little as 25 minutes* is the new standard



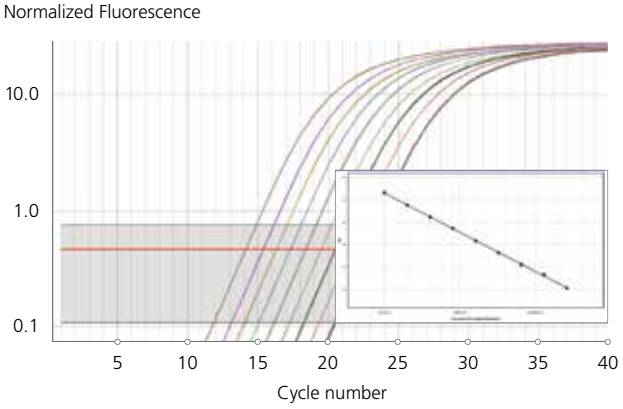
3.8 5 point, 2x dilution series of Hepatitis B virus (HBV) cDNA template. Starting amount of 3E+06 copies (n = 4 each); Efficiency = 90% (standard curve method); R^2 = 0.99; Time to complete run (including melt) = 26 min

*25 minute cycle times obtained with fast cycling master mixes and short amplicon assay designs targeting cDNA

Unrivaled Performance

Detect two-fold differences

- Confidently detect small differences
- High thermal uniformity and reproducibility
- Detect differences within a single cycle



3.9 Manganese superoxide dismutase gene (MnSOD). Eight point, 2x dilution series of human genomic DNA (n = 4 each); Efficiency = 98% (standard curve method); R^2 = 1.00

ORDER INFO

Product Name	Quantabio Catalog Number	Size
Q 2-channel qPCR Instrument	95900-2C	1 instrument
Q 4-channel qPCR Instrument	95900-4C	1 instrument
Q Tubes & Caps (20 racks/box, total of 960 tubes and caps)	95910-20	1 box

Q Cycler does not require the use of reference dyes.

AccuStart Genotyping ToughMix

AccuStart Genotyping ToughMix enables probe-based genetic analysis (SNP detection and allelic discrimination) directly from crude extracts, DBS punches, plant tissue and clinical specimens

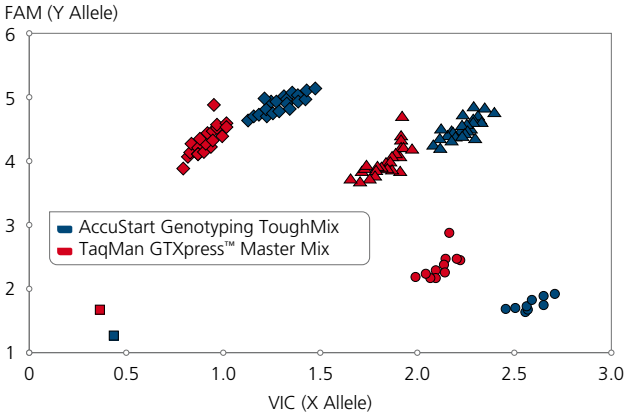
FEATURES AND BENEFITS:

- Optimized buffer chemistry destabilizes single base-pair mismatch probes, providing superior allelic discrimination and improved cluster separation for critical, single-nucleotide polymorphism (SNP) detection assays
- Sensitive, precise detection with ultrapure AccuStart II enzyme technology – maximum-yielding Taq DNA polymerase mutant controlled by stringent, multi-epitope antibody hotstart
- ToughMix reagent technology neutralizes a broad spectrum of PCR inhibitors common in plant and animal tissues, environmental sources, clinical specimens and complex food matrices
- Easy-to-use 2x concentrated SuperMix with AccuVue plate loading dye and pre-blended passive reference dye simplifies reaction setup
- Supports efficient vortex mixing with proprietary anti-foaming technology

DESCRIPTION:

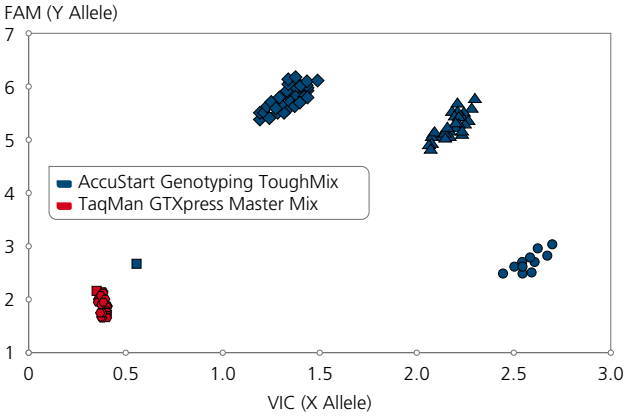
Genotyping ToughMix is a 1-tube qPCR SuperMix reagent compatible with all dual-label (hydrolysis) probe chemistries for both fast and conventional PCR cycling protocols or instruments. This proprietary formulation has been rigorously optimized to destabilize single base-pair mismatches to ensure precise allelic discrimination and cluster separation with SNP detection assays. The reagent is provided as a 2x concentrated ready-to-use reaction cocktail that contains all required reaction components, except primers, probe(s), and DNA template. Inert AccuVue plate loading dye helps to minimize pipette error and provides visual confirmation of thorough mixing.

ToughMix vs Competitor



4.1 Comparison to conventional master mixes AccuStart Genotyping ToughMix stands up to the challenge where other genotyping master mixes fall apart. ToughMix can be used with clean templates where it generates higher fluorescent signal and tighter clusters than the leading competitors.

Influence of PCR inhibitor



4.2 Comparison to conventional master mixes. In the presence of a common PCR inhibitor, humic acid (50 ng/µl), the competitors system is completely shut down while ToughMix delivers robust, accurate results

ORDER INFO

Product Name	Quantabio Catalog Number	Size
Genotyping ToughMix - 250 R	95115-250	250 x 20 µl rxns (2 x 1.25 ml)
Genotyping ToughMix - 1250 R	95115-012	1250 x 20 µl rxns (10 x 1.25 ml)
Genotyping ToughMix - 5000 R	95115-05K	5000 x 20 µl rxns (1 x 50 ml)
Genotyping ToughMix, ROX - 250 R	95116-250	250 x 20 µl rxns (2 x 1.25 ml)
Genotyping ToughMix, ROX - 1250 R	95116-012	1250 x 20 µl rxns (10 x 1.25 ml)
Genotyping ToughMix, ROX - 5000 R	95116-05K	5000 x 20 µl rxns (1 x 50 ml)
Genotyping ToughMix, Low ROX - 250 R	95117-250	250 x 20 µl rxns (2 x 1.25 ml)
Genotyping ToughMix, Low ROX - 1250 R	95117-012	1250 x 20 µl rxns (10 x 1.25 ml)
Genotyping ToughMix, Low ROX - 5000 R	95117-05K	5000 x 20 µl rxns (1 x 50 ml)

AccuStart II PCR Genotyping Kit

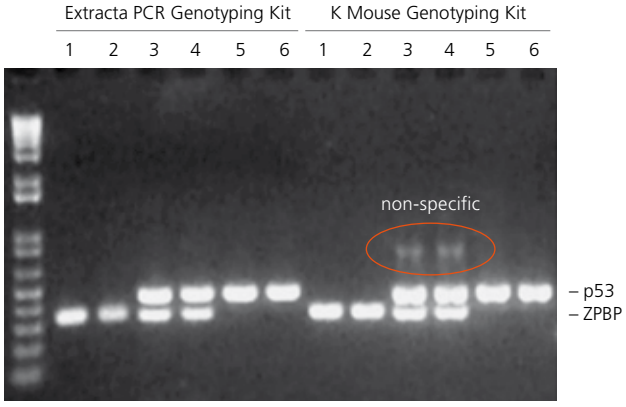
Completely reagent-based system enables reliable PCR genotyping with minimal pipetting skill

FEATURES AND BENEFITS:

- Premixed electrophoresis mobility loading dye reduces chances for post-PCR cross contamination
- Stabilized 2x PCR SuperMix enables convenient room-temperature setup
- High-yielding, ultrapure modified Taq DNA polymerase delivers robust, reliable duplex assay performance
- Stringent, ultrapure antibody hotstart ensures sensitive and specific target amplification
- Flexible protocol delivers rapid results in as little as 10 minutes

DESCRIPTION:

The AccuStart II Genotyping Kit is a complete reagent kit designed to support conventional, end-point PCR-based screening of transgenic animal models commonly used in life science research and is validated for use with mouse, fish, or insect tissue specimens. It combines a rapid, 2-component DNA extraction reagent with a user-friendly 2x concentrated PCR SuperMix with loading dye for seamless gel electrophoresis analysis. qPCR-grade genomic DNA template is obtained with minimal extraction volumes (≤ 100 µl) and can be carried out in ≤ 30-minutes on a standard PCR thermal cycler.



4.3 Two mouse tail snips (2.5 mm) were extracted according to the recommended conditions for each kit. The volume of each extract was brought to 300 µl and diluted 1/20 with TE buffer. 5 µl of diluted extract was used in a 25 µl PCR reactions.

Contents

- Extracta DNA Prep for PCR - Tissue, 2 x 25 ml Extraction Reagent and 2 x 25 ml Stabilization Buffer
- AccuStart II GelTrack PCR SuperMix (2x)
- 5 x 1.25 ml of 2X reaction mixture containing optimized concentrations of $MgCl_2$, dNTPs, AccuStart II Taq DNA Polymerase, AccuStart Taq antibodies, reaction buffer, stabilizers and gel loading dyes. Individual components can be reordered separately.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
AccuStart II Genotyping Kit - 100 R	95135-100	100 x 25 µl rxns
AccuStart II Genotyping Kit - 500 R	95135-500	500 x 25 µl rxns

AccuMelt HRM SuperMix

AccuMelt HRM SuperMix maximizes differences in melt temperature and curve shape to allow discrimination of DNA sequence differences amongst different samples

FEATURES AND BENEFITS:

- See sequence differences clearly – robust amplification ensures sufficient yield of products to generate discrete melt curves
- Accurate genotype calling – comparable or better performance than TaqMan Genotyping
- Work with rare or precious samples – large range of template inputs possible
- Specificity – works with lower Mg^{2+} concentration than other systems thus enhancing assay accuracy

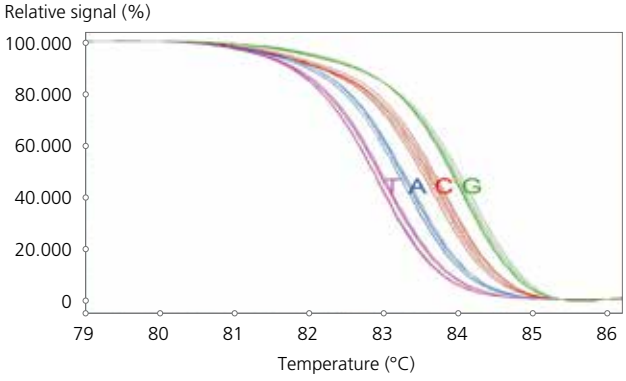
DESCRIPTION:

AccuMelt HRM SuperMix

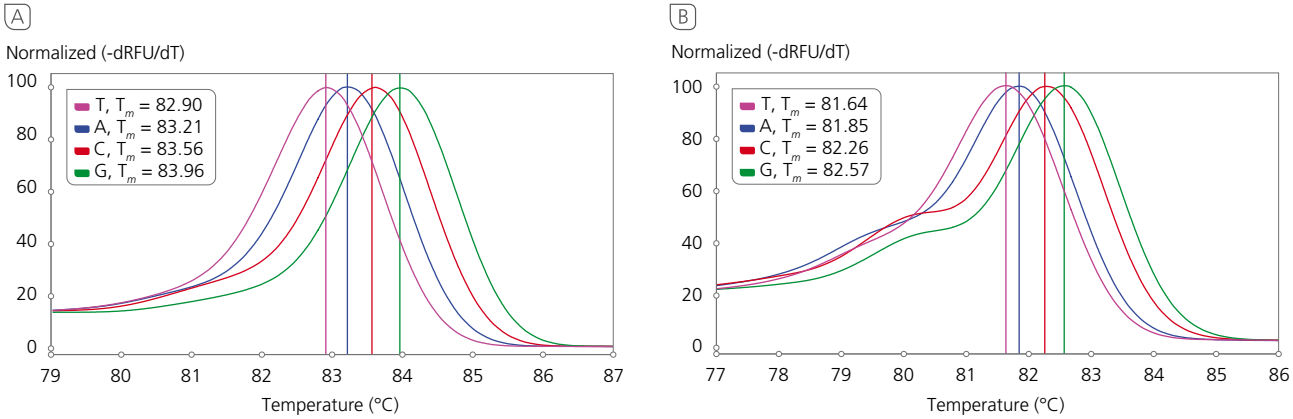
AccuMelt HRM SuperMix is a ready-to-use 2X concentrated hot-start PCR mix containing SYTO 9™ green fluorescent DNA-binding dye.

Superior Resolution of Genotypes

SNP Genotyping is a useful application for HRM and illustrates the capabilities of AccuMelt HRM SuperMix. Genotypes are readily identified based on unique melting profiles depending on a sample's sequence (Figure 1). Furthermore, AccuMelt HRM SuperMix gives superior resolution of difficult genotypes when compared to the leading competitor's mix based on greater T_m differences observed for A → T transversions (Figure 2).



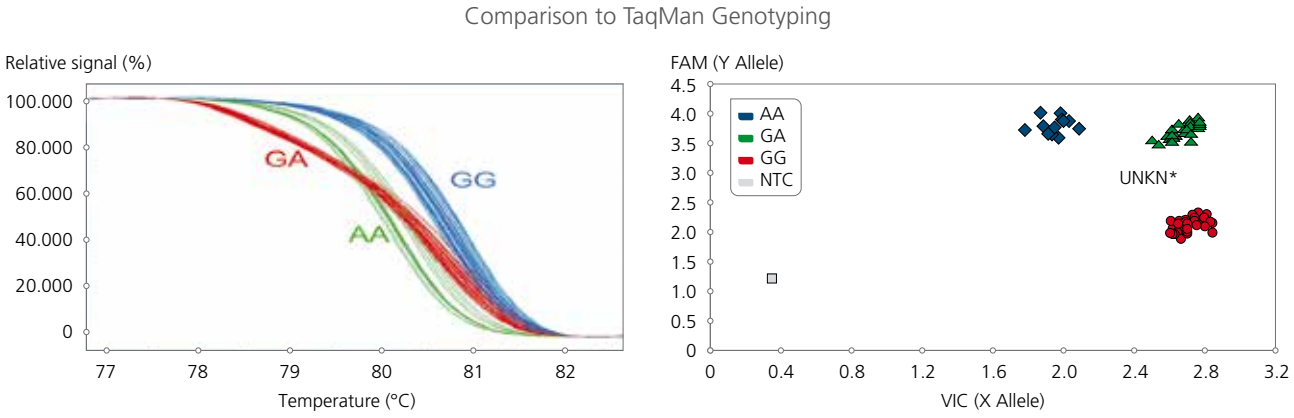
4.4 High resolution melting analysis of a model SNP system with a single A,C,G,or T variant base. AccuMelt HRM SuperMix readily resolves each genotype and T_m differences are easily visualized in normalized melting curve plots (Roche, Lightcycler 480).



4.5 Effect of T,A,C, or G variant base on T_m in a model HRM SNP system with either AccuMelt HRM SuperMix (Panel A), or a competitor's SYTO 9 dye master mix (Panel B). Plots of averaged melt peaks normalized to maximum signal for each system.

Comparison to TaqMan Genotyping

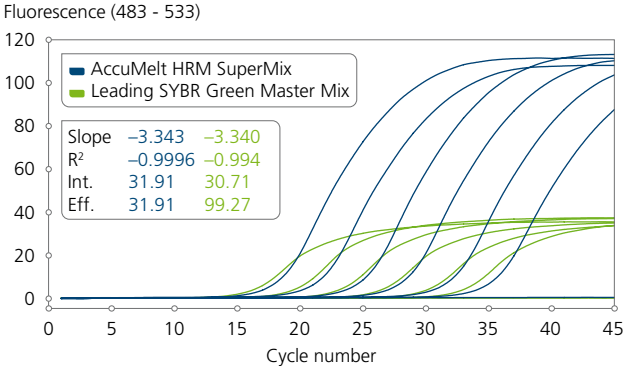
TaqMan Genotyping has been used successfully in SNP analysis and other allelic discrimination applications. This widely adopted standard in genotyping was used as a benchmark to assess the utility of HRM with our SuperMix. AccuMelt HRM was determined to be just as effective as TaqMan Genotyping in SNP analysis and was even able to call the genotype for a difficult sample which the TaqMan assay could not resolve.



4.6 Accuracy of HRM genotyping with AccuMelt HRM SuperMix was evaluated by comparison to TaqMan detection of the G>A rs1801133 SNP in the MTHFR gene (Panel A). HRM normalized melting curves (Panel B). TaqMan allelic discrimination plots. TaqMan failed to resolve Sample D3 (labeled as "UNKN") which was typed as a heterozygote by HRM.

Robust Amplification

Consistent robust amplification is critical to accuracy in HRM analysis. AccuMelt HRM SuperMix will drive all PCR amplifications to plateau regardless of the quantity of template input. This ensures accurate results regardless of the quantity of DNA available.



4.7 High yield, high efficiency PCR with AccuMelt HRM SuperMix. Real-time PCR of GAPDH was amplified from log-fold serial dilutions of qScript synthesized cDNA from HeLa cell total RNA (10 ng to 0.1 pg) was carried out with either a leading SYBR Green Master Mix or AccuMelt HRM SuperMix using the following cycling conditions: 95°C, 20 s; followed by 45 cycles of: 95°C, 3 s; 60°C, 20 s. Averaged plots for quadruplicate reactions for each input quantity are shown.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
AccuMelt HRM SuperMix - 250 R	95103-250	250 x 20 µl rxns (2 x 1.25 ml)
AccuMelt HRM SuperMix - 1250 R	95103-012	1250 x 20 µl rxns (10 x 1.25 ml)

5.0 microRNA Profiling

Sensitive, precise quantification of microRNAs using optimized reagent solutions and economical dye-based qPCR detection

The qScript microRNA cDNA Synthesis Kit is optimized to provide highly-sensitive reverse transcription of small, non-coding RNA and is compatible with either total RNA samples or miRNA-enriched template. This optimized kit provides reagent components for polyadenylation of small non-coding RNAs and first-strand cDNA synthesis with qScript reverse transcriptase. A novel sequence is incorporated by the oligo(dT) primer that can be utilized for qPCR quantification using the PerfeCta Universal microRNA Primer and miR-specific forward assay primer (PerfeCta miR Assay). The kit comes complete with a SNORD44 Human Positive Control Primer and extra Poly(A) Tailing Buffer and MicroRNA cDNA Reaction Mix to enable minus poly(A) polymerase control reactions to enable precise control for signal contributions from precursor pri-microRNA.

PerfeCta SYBR Green SuperMixes contain ultrapure, high-yielding Taq with stringent antibody hotstart and maximum SYBR Green dye content to enable sensitive and precise DNA amplification of small PCR amplicons (e.g. microRNA templated cDNA).

qScript microRNA cDNA Synthesis Kit

Reverse transcribe small, single-stranded RNAs with superior sensitivity

FEATURES AND BENEFITS:

- Log-fold greater sensitivity than inefficient stem-loop priming methods.
- Broad linear dynamic detection range across a range of RNA inputs (10 pg – 1 µg total RNA).
- Incorporate novel adapter sequence that can be utilized for qPCR quantification using PerfeCta Universal microRNA Primer

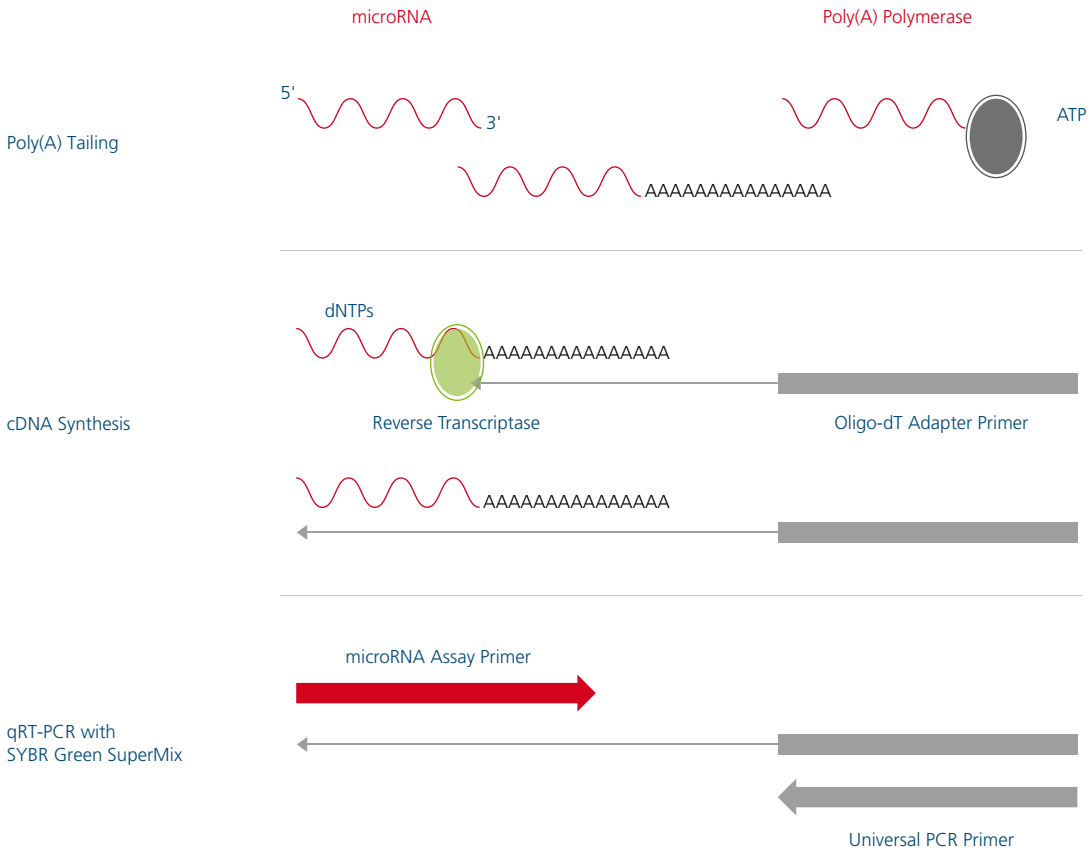
DESCRIPTION:

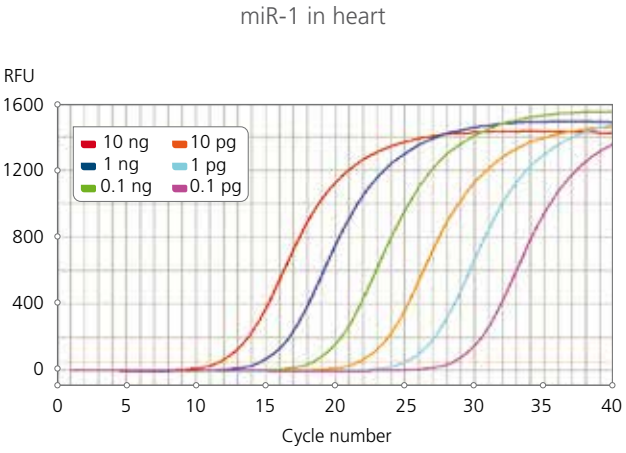
The qScript microRNA cDNA Synthesis Kit is an optimized reagent system that reverse transcribes small single-stranded RNA into 5'-labeled cDNA using either total RNA or miRNA enriched samples.

Single-stranded RNA is first polyadenylated by poly(A) polymerase before reverse transcription into universal cDNA using high performance qScript RT with a proprietary adapter oligo(dT) primer. The universal cDNA template enables simple and cost-effective microRNA profiling when used together with wet-lab validated PerfeCta microRNA Assays, PerfeCta Universal PCR Primer and PerfeCta SYBR Green SuperMix.

This complete kit includes positive (human) control primer, SNORD44, that can be used to quantitate ubiquitously expressed small nucleolar RNA. In addition, the kit contains 20% extra poly(A) polymerase reaction buffer and microRNA cDNA Reaction Mix to perform (-) poly(A) polymerase and (-) reverse transcriptase control reactions.

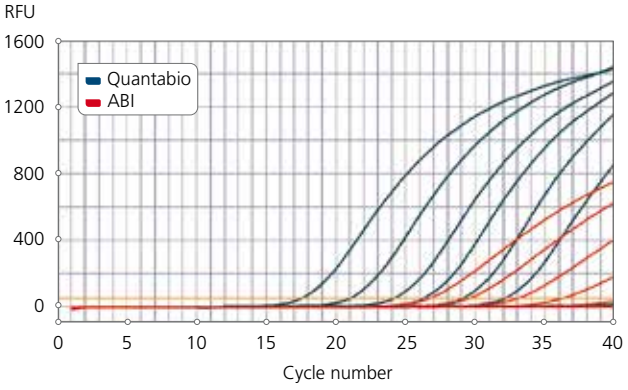
Quantabio's qScript microRNA system



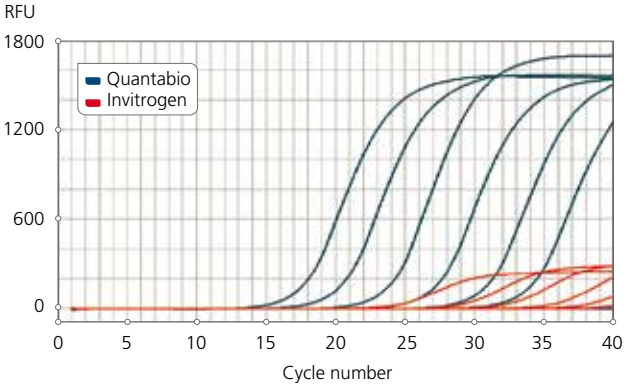


5.1 Detection of Rare miRNAs. microRNAs Quantabio's microRNA profiling system provides linear detection and quantification of miRNAs across total-RNA input levels spanning six orders of magnitude. This means miRNAs will be detected even when tissue is scarce or the miRNAs are rare. The Quantabio miRNA system will detect low copy miRNAs more reliably than other systems and due to the minus-PAP control you will have confidence in the results.

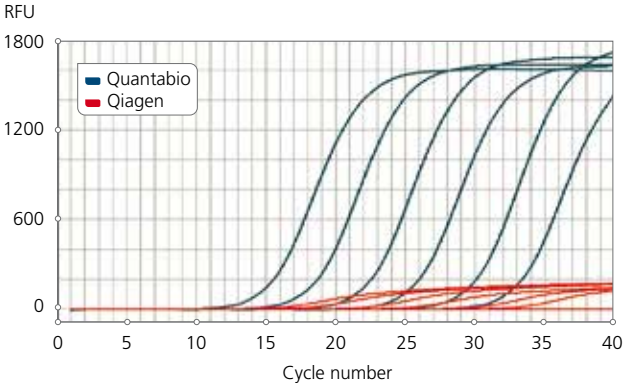
miR-124a



miR-27a



Let 7a



5.2 **Superior Sensitivity.** Quantabio's miRNA Profiling System yields superior results when compared to other leading miRNA quantification systems. The qScript microRNA cDNA Synthesis Kit, PerfeCTa microRNA-specific assays and PerfeCTa SYBR Green SuperMix form an integrated system that yields industry-leading results.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
qScript microRNA cDNA Synthesis Kit - 25 R	95107-025	25 x 20 µl rxns
qScript microRNA cDNA Synthesis Kit - 100 R	95107-100	100 x 20 µl rxns

Primer design using the qScript miRNA Quantification System

Basic Steps for Primer Design

1. Convert miRNA sequence to a DNA sequence
2. Append the reverse complement of the oligo-dT adapter primer (sequence provided below) to its 3' end.
3. Using primer design software or web-based tool, design the miRNA-specific FORWARD PRIMER that is compatible and T_m-balanced with the REVERSE PRIMER (universal primer, sequence provided below).

Example – human miR-193P

1.

miRNA sequence:

5'-UGUGCAAAUCUAUGCAAAACUGA-3'

↓ Convert to DNA sequence

5'-TGTGCAAATCTATGCAAAACTGA-3'

2.

Oligo dT Adapter Primer:

5'-GCATAGACCTGAATGGCGGTAAGGGTGTGGTAGGCGAGACATTTTTTTTTTTTTTTTTTT-3'

↓ Make reverse complement

5'-AAAAAAAAAAAAAAAAAAAATGTCTCGCCTACCACACCCTTACCGCCATTCAGGTCTATGC-3'

Template created by appending the RC of adapter primer to miRNA (DNA seq):

5'-TGTGCAAATCTATGCAAAACTGAAAAAAAAAAAAAAAAAAAAATGTCTCGCCTACCACACCCTTACCGCCATTCAGGTCTATGC-3'

3.

Design FORWARD PRIMER

TGTGCAAATCTATGCAAAACTGA →

5'-TGTGCAAATCTATGCAAAACTGAAAAAAAAAAAAAAAAAAAAATGTCTCGCCTACCACACCCTTACCGCCATTCAGGTCTATGC-3'

← ATGGCGGTAAGTCCAGATACG

Select last 21 bases as REVERSE PRIMER (Universal Primer)

When designing the forward primer, specific to your miRNA, restrict the search to 1-25 bases. In the example above the software (Oligo7) determined the optimal primer sequence (green) to be in effect the miRNA sequence. In other cases, depending on T_m balancing and compatibility with the universal primer, it may be longer or shorter.

Once you have designed the your miR-specific forward primer, you can order it through the oligonucleotide vendor of your choice. The reverse (universal) primer is included with the qScript microRNA cDNA synthesis kit. The experimental protocol for using the primers is available at **www.quantabio.com/resources**, along with protocols for the qScript microRNA cDNA synthesis kit and PerfeCTa SYBR Green SuperMix.

Developed Assays

A list of primers for more than 1500 miRNAs have already been designed and are available for download at **www.quantabio.com/products/microrna-profiling**. If you are a customer who previously purchased our PerfeCTa microRNA Assays, you will find the sequence for the assays of interest in this document. Use the ctrl-F function within excel to search by ID, accession number, or miRNA sequence.

6.0 Next Generation Sequencing (NGS)

sparQ DNA Frag & Library Prep Kit

Reliable enzymatic fragmentation safeguards samples for sequencing success

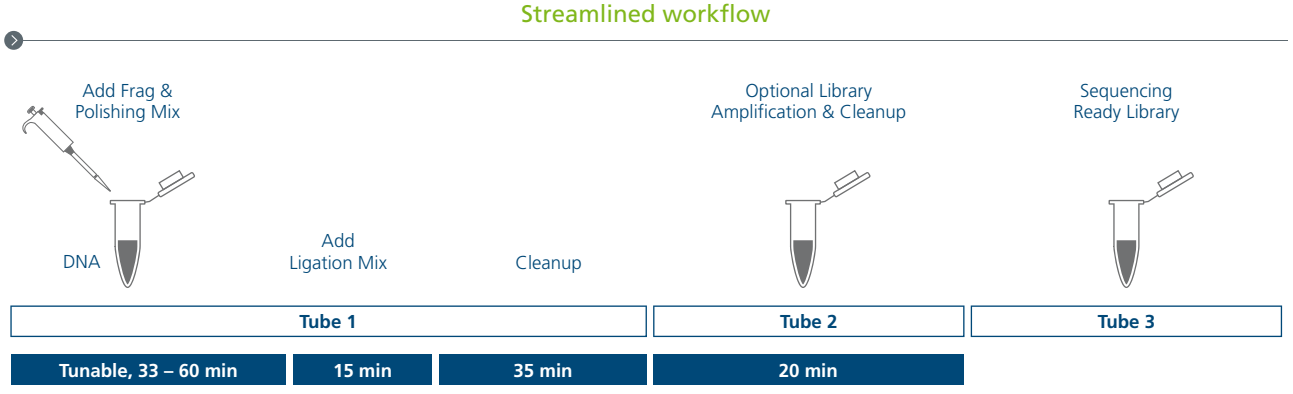
FEATURES AND BENEFITS:

- Simple 2 step workflow employs a unique enzyme mix safeguarding samples from over fragmentation
- Tunable and reproducible fragmentation profiles from 100 bp to 800 bp across a range of sample types
- Flexible generation of high quality libraries from 1 ng – 1 µg of input DNA
- PCR-free workflow enabled from 100 ng
- Minimized bias across challenging regions for improved sequencing results

DESCRIPTION:

The sparQ DNA Frag & Library Prep Kit optimizes the integration of enzymatic fragmentation into a two-step protocol for the streamlined construction of libraries for sequencing on Illumina NGS platforms. A single tube enzyme mix facilitates the combination of fragmentation and DNA polishing reactions minimizing over fragmentation while greatly simplifying library prep.

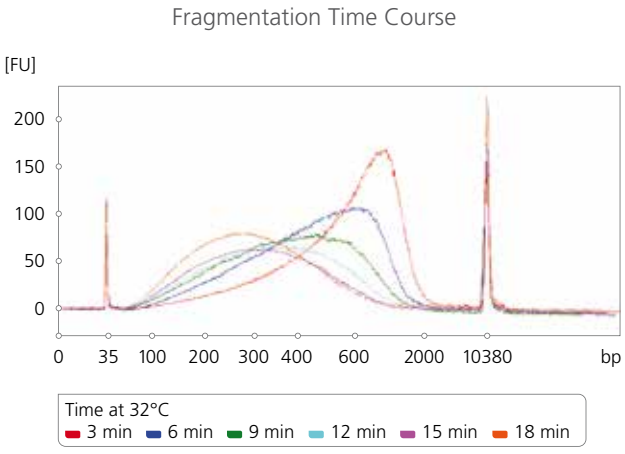
Quantabio's engineered DNA frag and polishing enzymes work in concert generating fragment sizes that are tunable and reproducible based on reaction time. Double-stranded DNA molecules are fragmented followed by DNA polishing reactions where 5'-phosphorylated and 3'-dA-tailed DNA fragments suitable for direct ligation of sequencing adapters are generated. Subsequent ligation of sequencing adapters is performed without an intervening cleanup step. The streamlined workflow can be completed in under 3 hours with minimal hands-on time accommodating DNA input amounts from 1 ng to 1000 ng. The HiFi PCR Master Mix and Primer Mix allow unbiased amplification of fragments with appropriate adapters ligated to both ends. PCR-Free workflows are enabled from 100 ng of starting material.



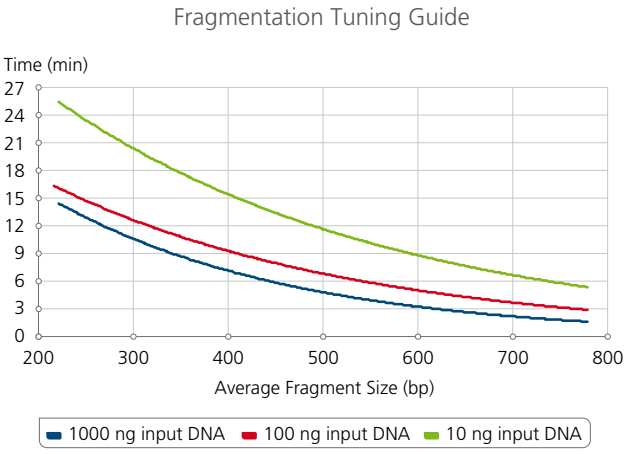
6.9 **The streamlined workflow utilizes a proprietary enzyme mix that integrates tunable and reproducible fragmentation with DNA polishing simplifying library construction and minimizing over fragmentation.** The same single reaction tube is used to proceed to adapter ligation and cleanup, minimizing sample transfer steps. A second tube is used for workflows requiring PCR amplification, and a final tube receives the sequencing-ready library.

Tunable & reproducible fragmentation

The sparQ DNA Frag & Library Prep kit is designed to produce fragments that are tunable to application specific sizes. Flexible input DNA amounts range from 1 ng – 1 µg. The single tube enzyme mix fragments DNA and then automatically proceeds to the DNA polishing reaction minimizing potential over fragmentation. sparQ DNA Frag & Library Prep kit consistently produces target fragments aligned to the desired target size.



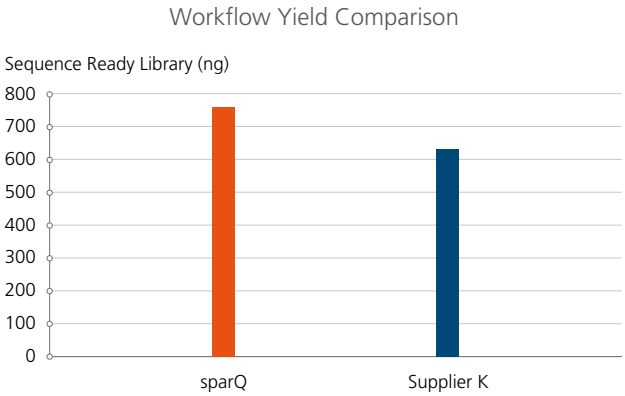
6.10 sparQ DNA Frag & Library Prep Kit is tunable to the desired fragment size. 100 ng Human gDNA was subjected to fragmentation with a series of incubation time points (3 – 18 min). After fragmentation, DNA samples were purified and then visualized using Agilent High Sensitivity DNA Kit.



6.11 Simply select the desired fragment size and input DNA amount. If input DNA falls between values displayed on the graph, an estimate can be used for optimizing fragmentation times.

Superior quality libraries & yields

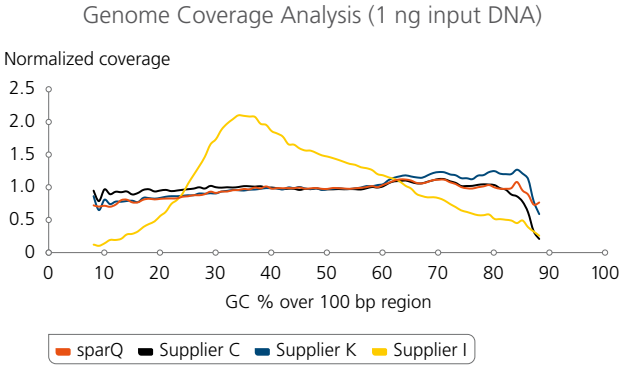
Designed to combine the highest efficiency enzymes for fragmentation, polishing, adapter ligation, and amplification, the sparQ enzymes generate superior yields of sequencing ready libraries over a broad range of input DNA. PCR-Free workflows are enabled for 100 ng input DNA. For applications requiring amplification, the high-fidelity master mix allows researchers to reduce the number of PCR cycles required to achieve the target concentration thereby reducing additional PCR-derived artifacts. Ultimately, precious samples can be conserved and reserved for additional applications when necessary.

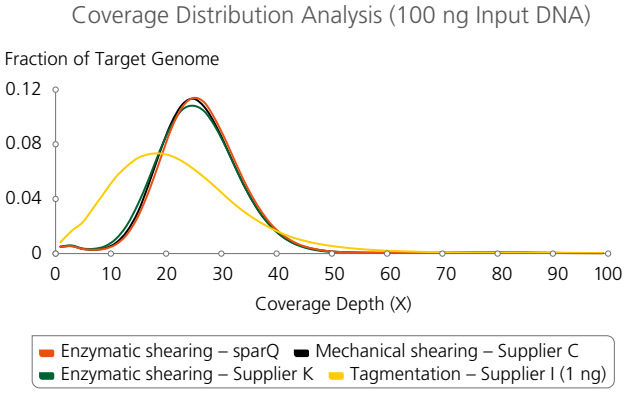


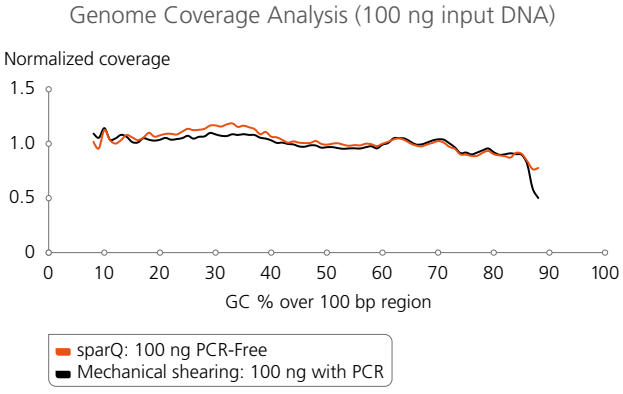
6.12 sparQ DNA Frag & Library Prep Kit shows significantly higher NGS library preparation efficiency. Libraries with 300 bp average DNA fragments from 100 ng of gDNA Coriell NA12878 were prepared using sparQ DNA Frag & Library Prep Kit and a commercial kit. Manufactures' manuals were carefully followed. Amplified libraries (5 cycles of amplification) were quantified by Qubit fluorometric quantitation method.

Minimize bias, maximize coverage uniformity to maintain genome diversity across a wide GC spectrum

The highly efficient library prep reduces coverage bias resulting in superior quality needed and expected, to minimize coverage gaps especially for challenging regions like GC- and AT-rich sequences. Reproducible and uniform genome coverage is achieved independent of input DNA amounts and closely resembles coverage obtained using mechanical shearing workflows resulting in optimized sequencing outcomes.







6.13 **Library prepared using sparQ DNA Frag & Library Prep Kit resulted in uniform coverage across the wide range of GC-content.** Libraries were prepared using different DNA fragmentation and library preparation kits with 1 ng or 100 ng of microbial gDNA followed by sequencing on Illumina MiSeq. 2 million reads from each tested library were down-sampled and analyzed. Coverage uniformity against GC-content bias resulting from different DNA fragmentation and library preparation kits were compared by plotting normalized coverage for a wide GC-content.
Libraries prepared using PCR-free workflow of sparQ DNA Frag & Library Prep Kit with 100 ng of microbial genomic DNA shows similar high performance as a typical amplified library prepared by Covaris mechanical shearing method.

Quality sequencing metrics and improved workflow economics

Optimized efficiencies in an enzymatic fragmentation 2 step protocol achieves quality sequencing metrics. The tunable fragmentation protocol safeguards samples from over fragmentation. A proprietary enzyme mix delivers high conversion efficiency and high yield of adapter ligated libraries. This allows lower input DNA amounts conserving precious sample. Finally, high fidelity amplification produces superior yields enabling fewer PCR cycles thus minimizing concerns for PCR introduced errors. The results are sequencing metrics with highly mappable reads and low duplication rates to ensure the greatest return on sequencing investment.

		1 ng input DNA		100 ng input DNA	
	Fragmentation	Mapped reads	Duplication	Mapped reads	Duplication
sparQ	Enzymatic	91.9%	0.07%	94.5%	0.04%
Supplier K	Enzymatic	92.4%	0.08%	93.5%	0.03%
Supplier I	Tagmentation	93.8%	0.28%	–	–
Supplier C	Mechanical	93.0%	0.09%	93.6%	0.03%

sparQ DNA Frag & Library Prep Kit generates high quality DNA libraries with minimal duplication artifacts. Libraries were prepared with 1 ng and 100 ng of microbial genomic DNA, amplified for 12 and 6 cycles respectively, and subsequently sequenced on Illumina MiSeq. Each library was down-sampled to 2 million reads (150 bp paired-end reads) and aligned to a reference genome with only unique alignments reported.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
sparQ DNA Frag & Library Prep Kit - 24	95194-024	24 rxns
sparQ DNA Frag & Library Prep Kit - 96	95194-096	96 rxns
Related Products		
sparQ Adapter Barcode Set A	95193-A96	12 single index barcoded for 96 rxns
sparQ Adapter Barcode Set B	95193-B96	12 single index barcoded for 96 rxns
sparQ HiFi PCR Master Mix	95192-050	50 rxns (1 x 1.25 ml)
sparQ HiFi PCR Master Mix	95192-250	250 rxns (5 x 1.25 ml)
PerfeCTa NGS Quantification Kit - Illumina - 500 R	95154-500	500 x 20 µl rxns
PerfeCTa NGS Quantification Kit - Illumina, ROX - 500 R	95155-500	500 x 20 µl rxns
PerfeCTa NGS Quantification Kit - Illumina, Low ROX - 500 R	95156-500	500 x 20 µl rxns

sparQ DNA Library Prep Kit

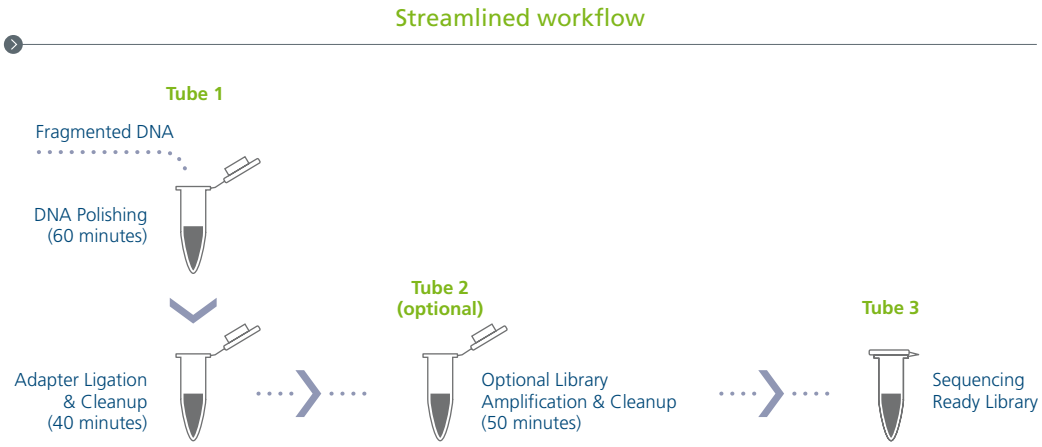
Prepare for sequencing success with the highest quality library

FEATURES AND BENEFITS:

- Simplified 2-step protocol speeds sample prep to under 3 hours and minimizes sample loss from transfer steps
- Increased library yields enable the construction of low input DNA sample from 250 pg
- Minimized bias improves coverage across difficult to sequence regions ensuring optimal results and reduced coverage gaps
- PCR-free workflows enabled from 100 ng input DNA
- Improved overall sequencing workflow economics

DESCRIPTION:

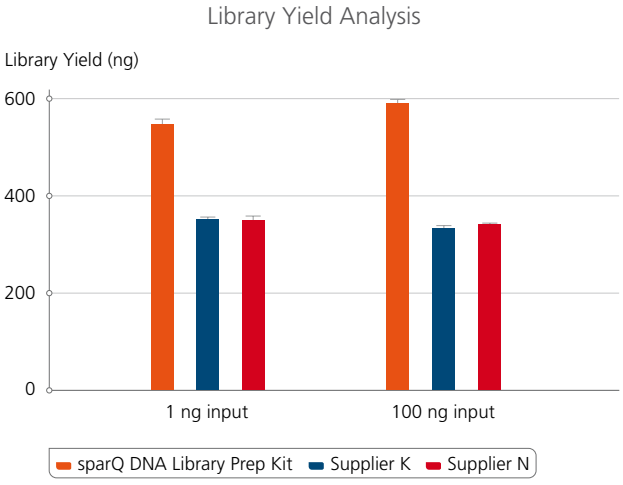
An optimized kit for the rapid construction of DNA libraries from fragmented double-stranded DNA for sequencing on Illumina NGS platforms. DNA polishing reactions are combined in a single step to convert fragmented DNA into 5'-phosphorylated and 3'-dA-tailed DNA fragments suitable for direct ligation of sequencing adapters without the need for an intervening cleanup saving valuable time and bead purification expense. The HiFi PCR Master Mix and Primer Mix allows for the optional, unbiased amplification of fragments with appropriate adapters ligated to both ends. The kit is compatible with input amounts from 250 pg to 1 µg DNA and multiple sample types.



6.5 The streamlined workflow can be completed in under 3 hours with minimal hands-on time. A single tube is used for DNA polishing, ligation, and cleanup. A second tube is used for workflows requiring PCR amplification and a final tube receives the sequencing-ready library.

Maximize library yields and increase sequenceable molecules

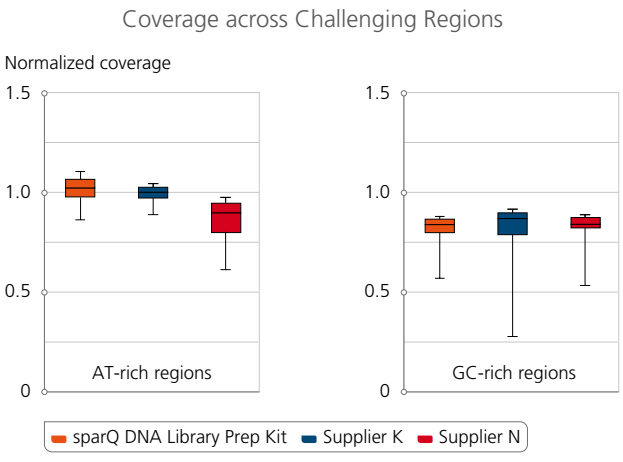
Critical first steps in library preparation depends heavily on the efficiency and sensitivity of the enzymes involved in DNA polishing and adapter ligation steps. The sparQ enzymes have been engineered, optimized, and selected for superior performance. The proprietary cocktail of enzymes is formulated to generate the highest yield and quality of adapter-ligated libraries over a broad range of input DNA down to as little as 250 pg enabling successful library construction for challenging samples and PCR-free workflows where input DNA is ≥100 ng.



6.6 sparQ DNA Library Prep Kit produces high quality libraries from a broad range of DNA inputs with significantly higher yields. Libraries were prepared with Covaris-sheared DNA (250 bp average size) using kit manufacturers' instructions. Amplified libraries (6 amplification cycles for 100 ng input DNA and 13 amplification cycles for 1 ng input DNA) were quantified with Qubit fluorometric quantitation method.

Minimize bias and gaps in sample genome coverage

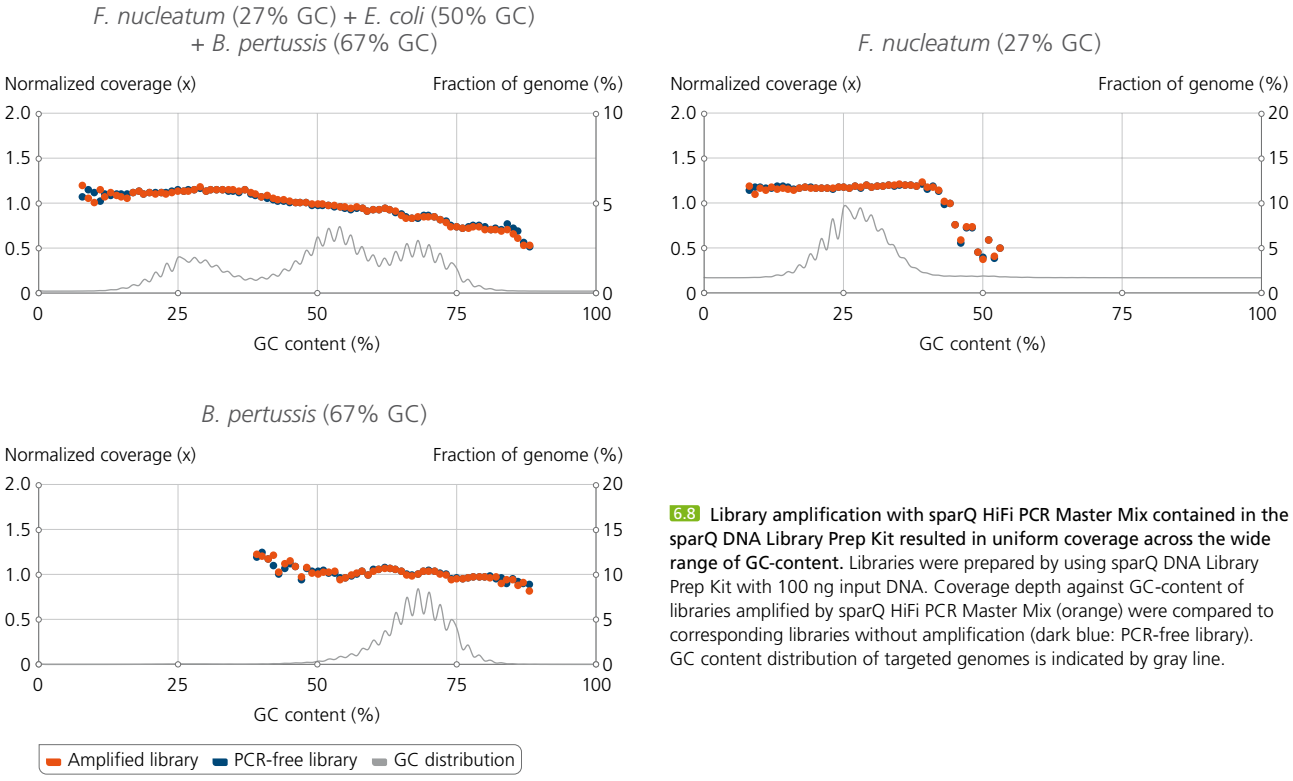
The highly efficient library prep reduces the bias resulting in the superior quality you need and expect, to minimize coverage gaps especially for challenging regions like GC- and AT-rich sequences. For applications requiring amplification, the high fidelity master mix is formulated to increase library yield reducing the number of cycles required to create a sequence-ready library thereby reducing additional PCR-derived artifacts.



6.7 Amplified libraries were prepared from 100 ng of microbial genomic DNA and subsequently sequenced on Illumina MiSeq. 2 million reads from each tested library were down-sampled and analyzed. Coverage uniformity for different library preparation kits were compared by plotting normalized coverage for both extreme AT-rich regions (8%-20% GC-content) and GC-rich regions (75%-88% GC-content).

Create amplified libraries with PCR-free results

Comparison of library preparation performed with sparQ DNA Library Prep Kit matches PCR-free workflows. Low amplification bias enables better coverage uniformity resulting in greater sequencing depth or multiplexing capabilities.



6.8 Library amplification with sparQ HiFi PCR Master Mix contained in the sparQ DNA Library Prep Kit resulted in uniform coverage across the wide range of GC-content. Libraries were prepared by using sparQ DNA Library Prep Kit with 100 ng input DNA. Coverage depth against GC-content of libraries amplified by sparQ HiFi PCR Master Mix (orange) were compared to corresponding libraries without amplification (dark blue: PCR-free library). GC content distribution of targeted genomes is indicated by gray line.

Improve Sequencing Results and Economics

High library conversion efficiency maintains a comprehensive view of your sample genome. Whether using an amplified or PCR-free workflow, sparQ DNA library prep produces industry leading sequencing results as determined by the high number of reads mapping back to the reference genome with minimal duplication rates. Outomes optimize your sequencing results and workflow economics.

	Library Amplification	1 ng input DNA		100 ng input DNA	
		Mapped reads	Duplication	Mapped reads	Duplication
sparQ	with Amplification	94.3%	0.07%	95.5%	0.04%
Supplier K	with Amplification	95.0%	0.09%	95.6%	0.04%
Supplier N	with Amplification	94.9%	0.07%	95.4%	0.03%
sparQ	PCR-Free			95.6%	0.03%
Supplier K	PCR-Free			95.3%	0.02%
Supplier N	PCR-Free			95.1%	0.02%

sparQ DNA Library Prep Kit generates high quality DNA libraries with minimal duplication rates. Libraries were prepared with 1 ng and 100 ng of microbial genomic DNA and subsequently sequenced on Illumina MiSeq. Each library was down-sampled to 2 million reads (150 bp paired-end reads) and aligned to a reference genome with only unique alignments reported.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
sparQ DNA Library Prep Kit - 24	95191-024	24 rxns
sparQ DNA Library Prep Kit - 96	95191-096	96 rxns
sparQ Adapter Barcode Set A	95193-A96	12 single index barcodes for 96 rxns
sparQ Adapter Barcode Set B	95193-B96	12 single index barcodes for 96 rxns
Related Products		
sparQ HiFi PCR Master Mix	95192-050	50 rxns (1 x 1.25 ml)
sparQ HiFi PCR Master Mix	95192-250	250 rxns (5 x 1.25 ml)
PerfeCta NGS Quantification Kit - Illumina - 500 R	95154-500	500 x 20 µl rxns
PerfeCta NGS Quantification Kit - Illumina, ROX - 500 R	95155-500	500 x 20 µl rxns
PerfeCta NGS Quantification Kit - Illumina, Low ROX - 500 R	95156-500	500 x 20 µl rxns

sparQ HiFi PCR Master Mix

Increasing efficiency and yield while lowering bias

FEATURES AND BENEFITS:

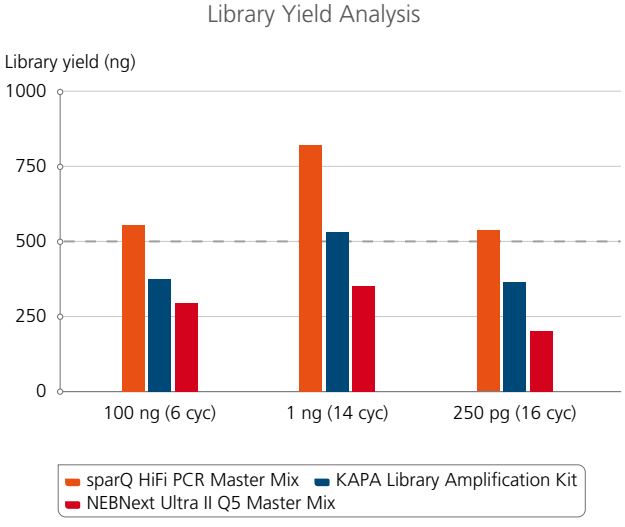
- Increased amplification efficiency results in higher yields for NGS library amplification even from low input DNA
- Unbiased amplification of DNA fragments provides improved coverage across AT- and GC-rich regions
- Improved overall sequencing economics

DESCRIPTION:

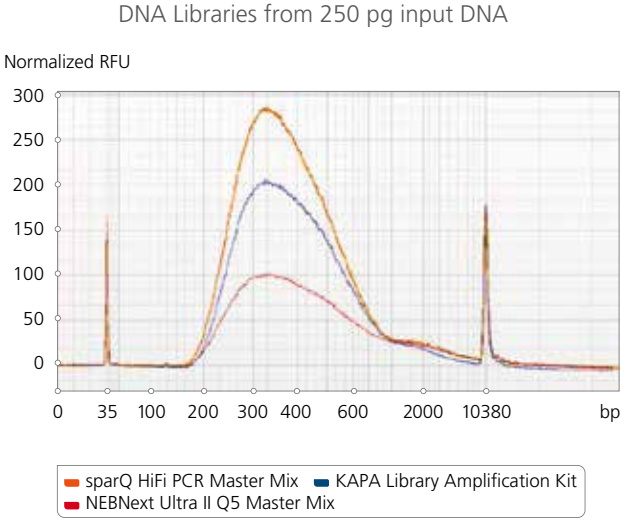
A high efficiency, high-fidelity, and low bias PCR master mix for NGS workflows requiring DNA library amplification prior to sequencing. The kit includes a primer mix allowing amplification of DNA libraries flanked by adapters containing the P5 and P7 Illumina® flow cell sequences.

Higher NGS library amplification efficiency

sparQ HiFi PCR Master Mix was specially designed for high efficiency library amplification from low DNA input. The master mix minimizes the number of amplification cycles needed to achieve the threshold required for sequencing. The result is a superior performing high fidelity master mix that increases library yield which reduces PCR-derived artifacts for a variety of sequencing applications.



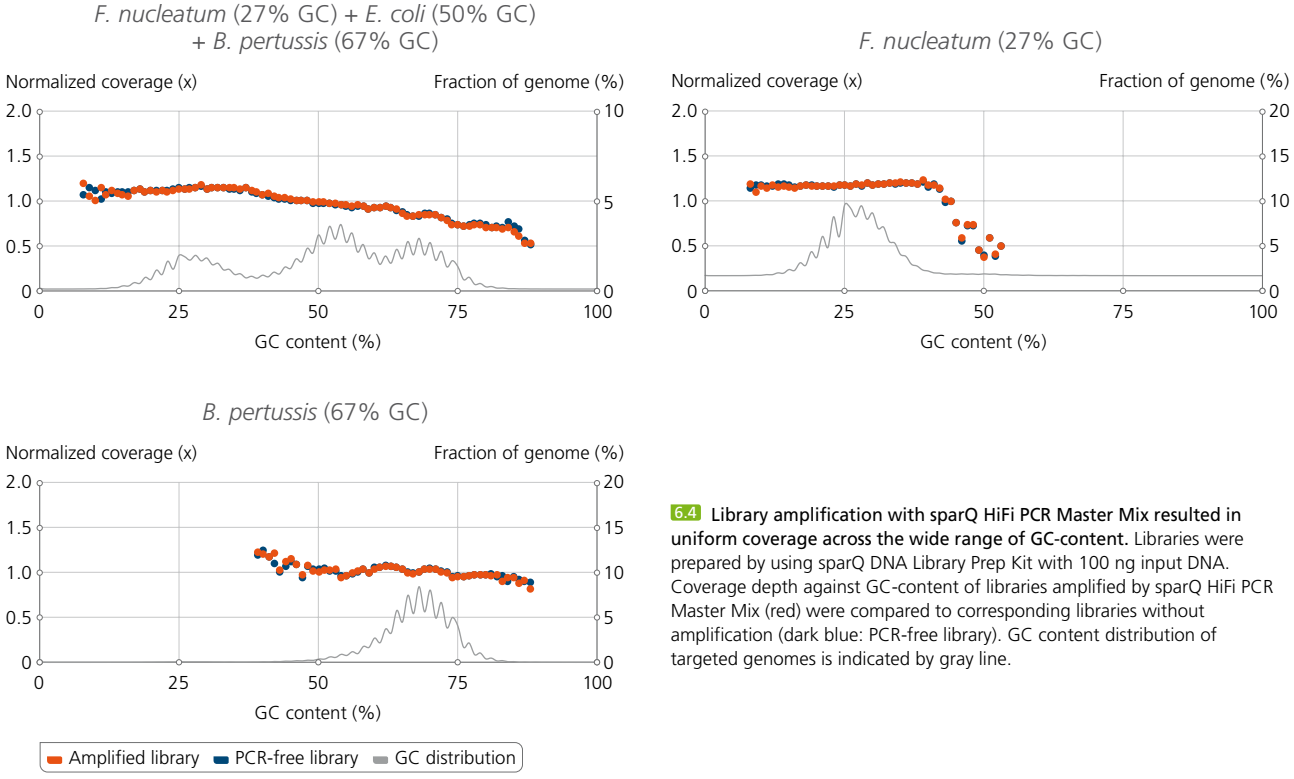
6.2 Libraries were prepared from Covaris-sheared DNA with sparQ DNA library prep kit prior to library amplification. Pre-amplified libraries were then amplified using sparQ HiFi PCR Master Mix (red), KAPA Library Amplification kit (blue), or NEBNext® Ultra™ II Q5® Master Mix (green) with the identical amplification cycle numbers (6 cycles for 100 ng input DNA, 14 cycles for 1 ng input DNA, and 16 cycles for 250 pg input DNA). Amplified libraries were quantified with Qubit fluorometric quantitation method and qPCR-based quantification method (data not shown).



6.3 The fragment size distribution and the quality of the amplified DNA libraries from 250 pg input DNA were analyzed using a high sensitivity DNA analysis kit on a Bioanalyzer. Libraries were amplified using sparQ HiFi PCR Master Mix (red), KAPA Library Amplification kit (blue), or NEBNext Ultra II Q5 Master Mix (green) with identical amplification cycle numbers (16 cycles for 250 pg input DNA).

Create amplified libraries with unbiased results

Comparison of library preparation performed with sparQ HiFi PCR Master Mix matches PCR-free workflows. Lower bias enables better coverage uniformity resulting in greater sequencing depth or multiplexing capabilities. Outcomes optimize your sequencing results and economics.



6.4 **Library amplification with sparQ HiFi PCR Master Mix resulted in uniform coverage across the wide range of GC-content.** Libraries were prepared by using sparQ DNA Library Prep Kit with 100 ng input DNA. Coverage depth against GC-content of libraries amplified by sparQ HiFi PCR Master Mix (red) were compared to corresponding libraries without amplification (dark blue: PCR-free library). GC content distribution of targeted genomes is indicated by gray line.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
sparQ HiFi PCR Master Mix - 50 R	95192-050	50 rxns (1 x 1.25 ml)
sparQ HiFi PCR Master Mix - 250 R	95192-250	250 rxns (5 x 1.25 ml)

PerfeCta NGS Library Quantification Kit

Real-Time qPCR is the most sensitive and precise method for quantifying adapter-ligated DNA molecules

PerfeCta NGS Library Quantification kits provide a comprehensive reagent solution in a single easy-to-use kit

FEATURES AND BENEFITS:

- Precise quantification of adapter-tagged library molecules enables maximum utilization of the flow cell
- Validated primer assays Illumina NGS platforms
- Titrated reference dyes for common real-time PCR instruments
- Stabilized, ready-to-use DNA standards are predefined for precise library quantification

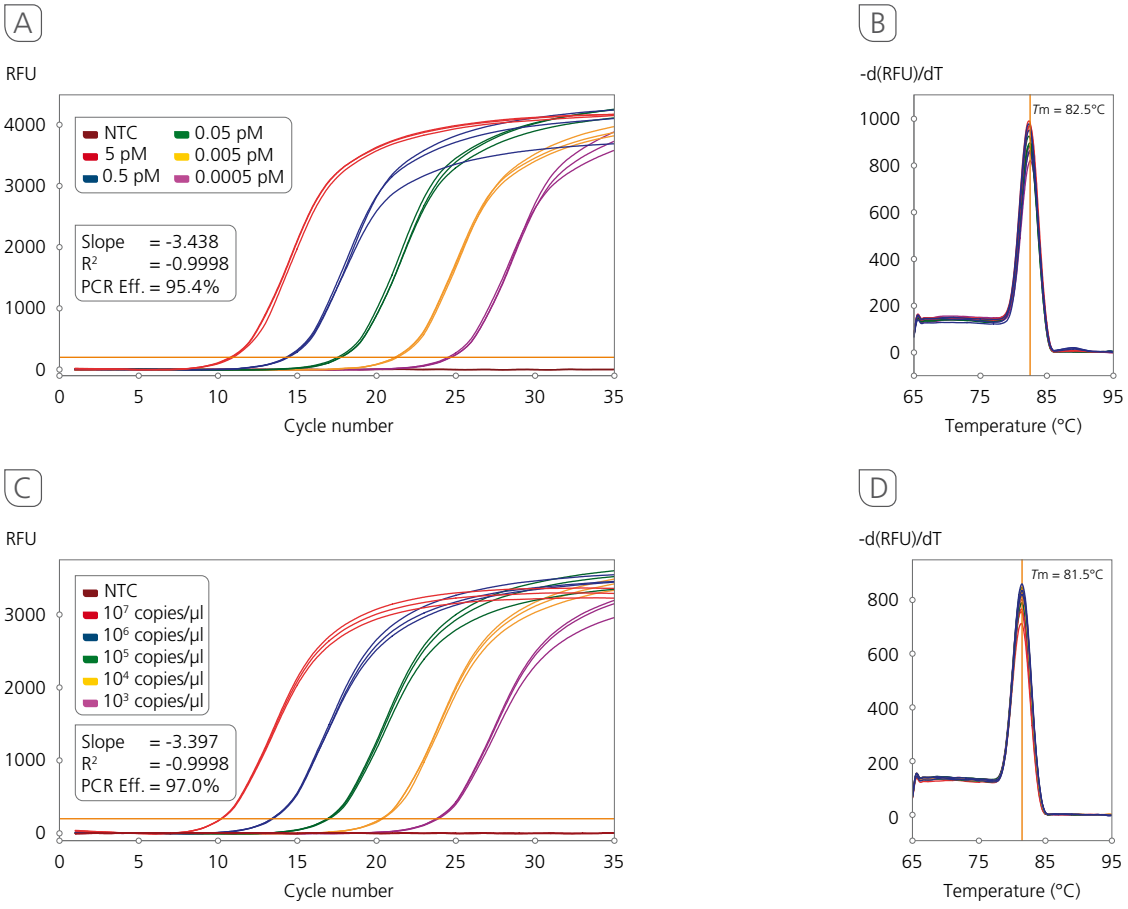
DESCRIPTION:

Accurate quantification of the number of amplifiable library molecules is the most critical step in the NGS workflow in obtaining high quality read data with next-generation sequencing technologies. The sparQ NGS Library Quantification Kit uses real-time PCR to specifically quantify library molecules that possess the appropriate adapter tag at each end. These are the suitable template molecules for Bridge PCR used for Illumina NGS platforms.

A common problem with some NGS library quantification protocols is the use of DNA standards that are too concentrated and generate qPCR data that are outside of the linear dynamic range for many qPCR instruments. Improper baseline settings result in compressions between the highest concentrated DNA standards that in turn give rise to inflated PCR efficiencies and inaccurate library quantification results. The NGS DNA standards supplied with the sparQ NGS Library quantification kits have been carefully selected to avoid these artifacts and produce NGS library standard curves with exceptionally high linear regression correlation coefficients.

PerfeCta NGS Quantification kits simplify the library quantification process by providing stabilized, pre-diluted standards, pre-qualified primer sets and an optimized dilution buffer for NGS library samples. This minimizes pipetting errors and ensures reproducible and precise qPCR results, even with dilute samples. The robust qPCR performance of PerfeCta SYBR Green SuperMix provides accurate quantification of NGS libraries with varying fragment sizes or GC content. Kits are available to support all major qPCR instrument platforms.

Illumina
The DNA standard for Illumina NGS platforms generates a 426 bp amplicon (48.8% GC). Primer sequences correspond to the "P5" and "P7" primer sequences for Illumina sequencing libraries:
Illumina forward primer: 5'-AAT GAT ACG GCG ACC ACC GA-3'
Illumina reverse primer: 5'-CAA GCA GAA GAC GGC ATA CGA-3'



6.1 **PerfeCta NGS library Quantification Kit performance data.** qPCR amplification of each of the five supplied DNA standards for Illumina NGS libraries (**panel A**) or Ion Torrent libraries (**panel C**) were carried out with the supplied primer sets (300 nM final concentration) and PerfeCta SYBR Green SuperMix in 20 µl reaction volumes on a Bio-Rad CFX-96. Reactions were incubated for 5 min at 95°C followed by 35 cycles of: 95°C, 10 s; 60°C, 20 s; 45 s, 72°C. Real-time fluorescence data was collected and analyzed at completion of the 72°C extension step. After completion of PCR, a dissociation (melt) curve was performed to verify amplification of a single specific product (**panels B and D**).

ORDER INFO

Product Name	Quantabio Catalog Number	Size
PerfeCta NGS Quantification Kit - Illumina - 500 R	95154-500	500 x 20 µl rxns
PerfeCta NGS Quantification Kit - Illumina, ROX - 500 R	95155-500	500 x 20 µl rxns
PerfeCta NGS Quantification Kit - Illumina, Low ROX - 500 R	95156-500	500 x 20 µl rxns

INSTRUMENT COMPATIBILITY

High Rox

- Applied Biosystems 5700
- Applied Biosystems 7000
- Applied Biosystems 7300
- Applied Biosystems 7700
- Applied Biosystems 7900
- Applied Biosystems 7900HT
- Applied Biosystems 7900HT Fast
- Applied Biosystems StepOne™
- Applied Biosystems StepOnePlus™

Low Rox

- Applied Biosystems 7500
- Applied Biosystems 7500 Fast
- Stratagene Mx3000P®
- Stratagene Mx3005P™
- Stratagene Mx4000™
- Applied Biosystems ViiA 7
- Applied Biosystems QuantStudio™
- Agilent AriaMx
- Douglas Scientific IntelliQube®

No Rox

- Q
- QIAGEN® Rotor-Gene® Q
- Bio-Rad CFX
- Other
- Roche Lightcycler

Bio-Rad iCycler iQ systems

- Bio-Rad iCycler iQ™
- Bio-Rad MyiQ™
- Bio-Rad iQ™ 5

DISCLAIMER / TRADEMARKS

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Version 3.0, 03/2018

MK-PC-0001 REV 01 Catalog Quantabio US 0918

Summary of Limited Label License Statements for Quantabio's PCR, qPCR, and cDNA synthesis kits

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