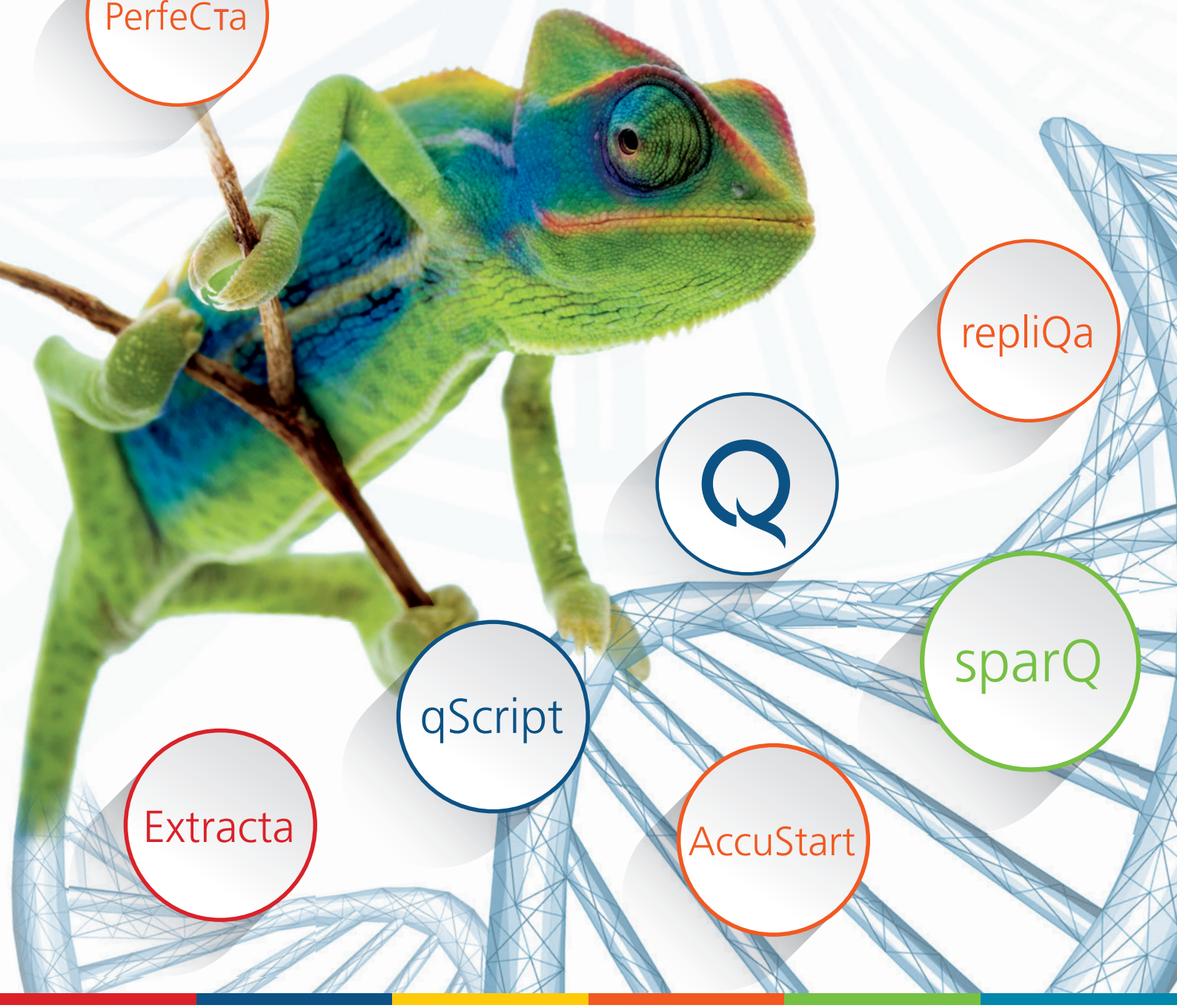
Quantabio

PRODUCT GUIDE



Sample Preparation | Reverse Transcription | PCR | Real-Time qPCR | NGS | qPCR Instrument

CONTENT

1.1 ABOUT QUANTABIO

A fast-growing, innovative brand and leading provider of advanced DNA and RNA amplification reagents for the most demanding molecular testing applications in applied, translational and life science research.

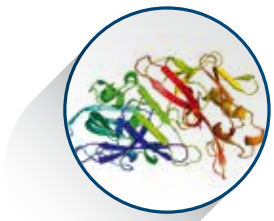
Our Recipe for Success

It's in our DNA. Be Resilient. ADAPT.

It starts from ideation with product design & development, continuing all the way to customer application support. We aim to make and ADAPT product solutions that deliver superior results, enable easier workflows and better affordability while providing unwavering support in ever changing life science and molecular diagnostic environments.

Your success is our success.

How we ADAPT product solutions in 4 Steps

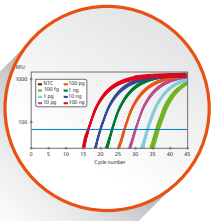


Step 1

Start with
Enzymes
Engineered for specific application

Step 2

Formulate with
Tough Additive
To optimize reagent compositions



Step 3

Create unique
Superior
Performing products

Applications

- RT
- qPCR
- PCR
- NGS

Step 4

Provide unweavering
Customer Support
For multiple molecular biology workflows

1.2 OUR HISTORY

Legacy of Innovation

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.

Founded 2001 by former amplification scientists from Life Technologies (Invitrogen)

Quanta Biosciences founded — **2001**

2002 – 2007
- qScript cDNA SuperMix
- PerfeCTa SuperMix
- PerfeCTa FastMix

2007 – 2012
- AccuMelt HRM
- qScript microRNA System
- PerfeCTa ToughMix
- qScript XLT Kit
- GelTrack SuperMix
- AccuStart II Taq DNA Polymerase

2012 – 2016
- qScript XLT Kit
- PerfeCTa Multiplex Kit
- PerfeCTa NSG Quantification Kit
- Ultraplex 1-Step RT-qPCR ToughMix
- Renamed to: Quantabio

2017
- repliQa HiFi Assembly Mix
- sparQ HiFi PCR Master Mix
- sparQ DNA Frag & Library Prep Kit
- sparQ DNA Library Prep

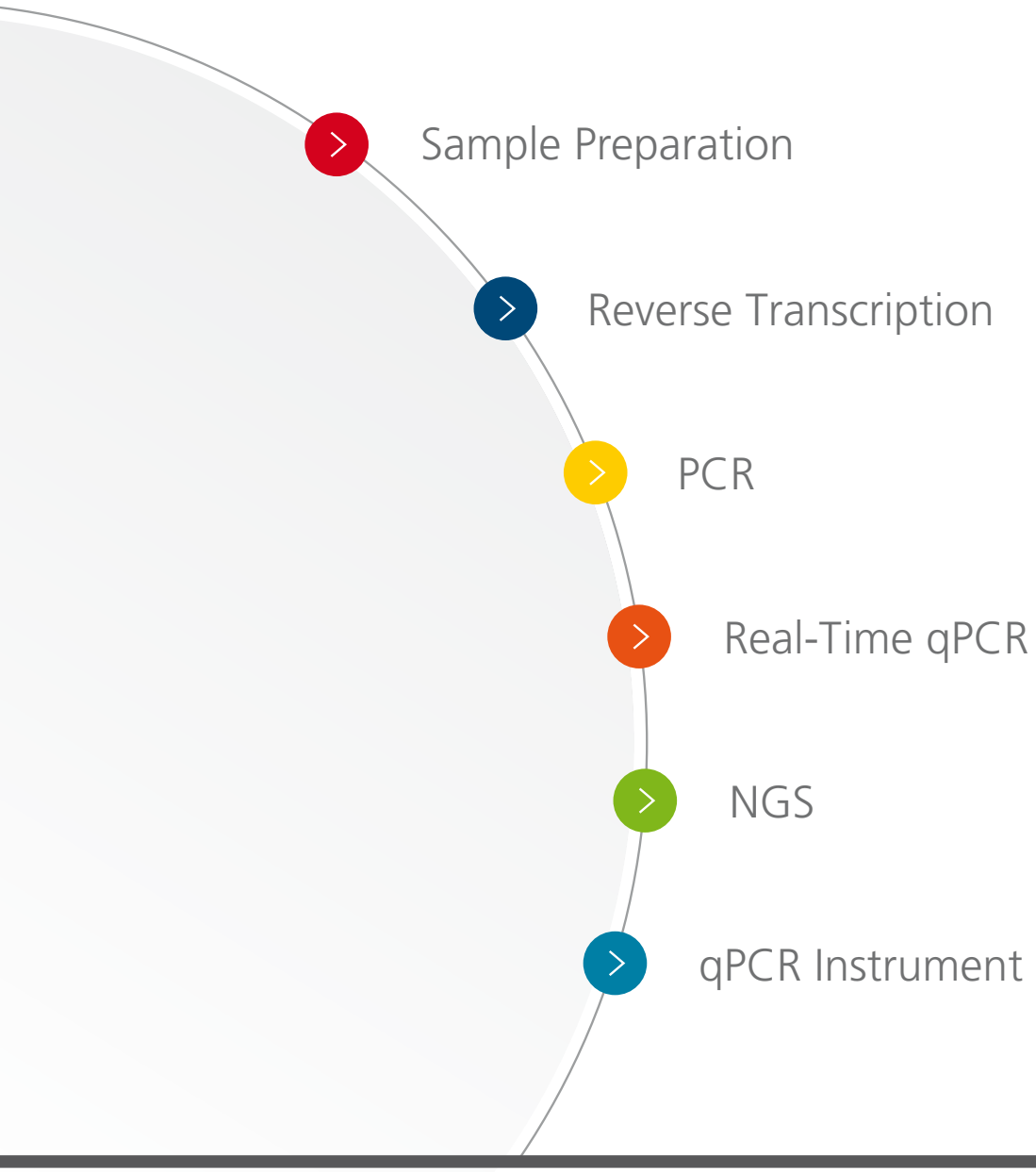
2018
- Q qPCR Cycler
- sparQ PureMag Beads

2019
- QScript lyo 1-step
- sparQ Fast Library Quant Kit
- AccuStart Long Range SuperMix

2020
- repliQa HiFi ToughMix
- AccuStart Long Range SuperMix
- qScript 1-Step Virus ToughMix
- sparQ Universal Library Quant Kit

1.3 MARKETS & APPLICATIONS

Quantabio utilizes it's novel DNA and RNA amplification technologies along with proprietary buffer chemistry to create unique and differentiated reagent solutions for life science applications ranging from sample extraction to cDNA synthesis, genotyping, gene expression, cloning and even next generation sequencing. Our products are used in thousands of labs around the world for life science research as well as in applied testing markets.



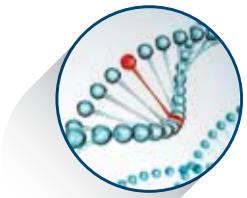
Translational Research Markets

Quantabio reagents are the gold standard for sensitive and reliable quantitative assay performance in PCR, qPCR and NGS. As an example, our proprietary ToughMix® reagents enable efficient amplification for challenging PCR inhibitors.

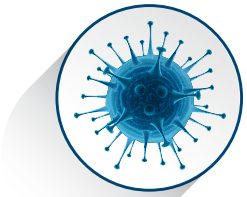
Quantabio products are used globally for detection of a variety of infectious diseases and in new born screening assays such as:

- Polio
- Influenza
- West Nile
- Zika
- Spinal Muscular Atrophy (SMA)
- Severe Combined Immunodeficiency (SCID)
- COVID-19

Genetic Mutation Detection



Pathogen Detection



Applied Markets

Quantabio products are used routinely for applications such as:

- GMO Testing
- Bacterial Contamination
- Beer Spoilage

Food Testing

Animal Health

Environmental Testing

Plant Testing

1.4 FAMILY OF BRANDS

Extracta

Extracta extraction reagents provide a simplified and cost-effective alternative to traditional nucleic acid (NA) purification methods and are optimized to work in series with ToughMix products. Optimized for clinical specimens (blood and dried blood spots), plant and animal tissues and environmental samples.

AccuStart

AccuStart ultrapure DNA polymerase, contains a stringent antibody hotstart to ensure specific and efficient primer extension only after activation at 94°C and rigorouslyigorously purified to remove host *E. coli* genomic DNA as residual host DNA in typical recombinant enzyme preparations can limit assay sensitivity and obscure detection of low copy targets.

PerfeCta

PerfeCta qPCR reagents combine a stringent, ultrapure antibody hotstart with performance engineered DNA polymerase in stabilized 1-tube formulations optimized for the specific performance needs of real-time quantitative PCR. Proprietary additives help eliminate persistent bubbles to enable efficient vortex mixing and fewer technical replicates thereby conserving precious sample. Adaptive buffer chemistry accommodates most assay designs and can be used with existing assay designs.

qScript

qScript reverse transcription reagents leverage proprietary, performance-engineered qScript reverse transcriptase in a variety of stabilized, user-friendly reagent formulations that maximize cDNA yield and provide linear cDNA synthesis across a broad dynamic range of input RNA. qScript cDNA synthesis kits redefine what is possible in speed, convenience, reproducibility, specificity and limit of detection (LOD) sensitivity in qPCR and RT-PCR applications.

repliQa

repliQa HiFi ToughMix provides superior speed and inhibitor tolerance for a variety of molecular biology applications. The high efficiency repliQa ToughMix is ideal for a range of genetic engineering applications, such as: cloning, site-directed mutagenesis and synthetic biology.

sparQ

sparQ offers complete solutions for library preparation, amplification, purification and quantification for Illumina next generation sequencing platforms. High quality reagents deliver unmatched efficiency and robust performance to ensure reliable and reproducible sequencing results while reducing total sequencing costs.

1.5 CORE 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 antibody 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

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 enhances 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 reliable assay 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

Quantabio is your trusted reagent supply partner. We pride ourselves on manufacturing quality excellence and an industry-leading technology portfolio.

2.0 Sample Preparation

Extracta DNA Prep

Quick and easy DNA extraction of PCR-ready genomic DNA

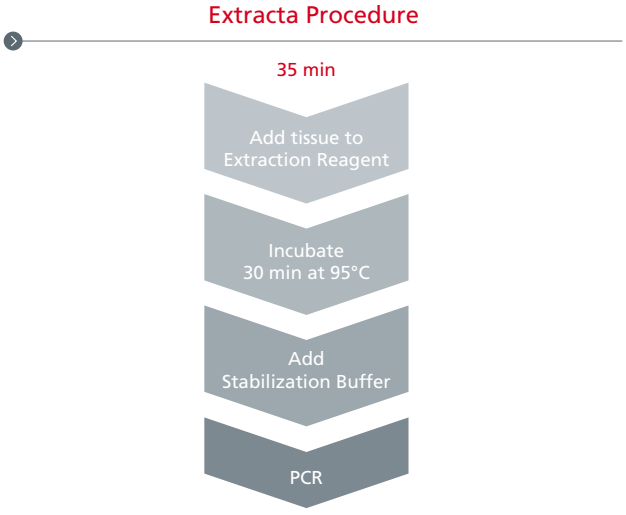
Extracta DNA Prep is an entirely reagent-based system for extracting and stabilizing template DNA from a variety of biological starting materials for sensitive downstream applications such as PCR, qPCR and HRM analysis

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 - 2.5 ml	95091-002	2.5 ml
Extracta DNA Prep - 25 ml	95091-025	25 ml
Extracta DNA Prep - 250 ml	95091-250	250 ml

Extracta DBS

PCR-ready genomic DNA from dried blood spots

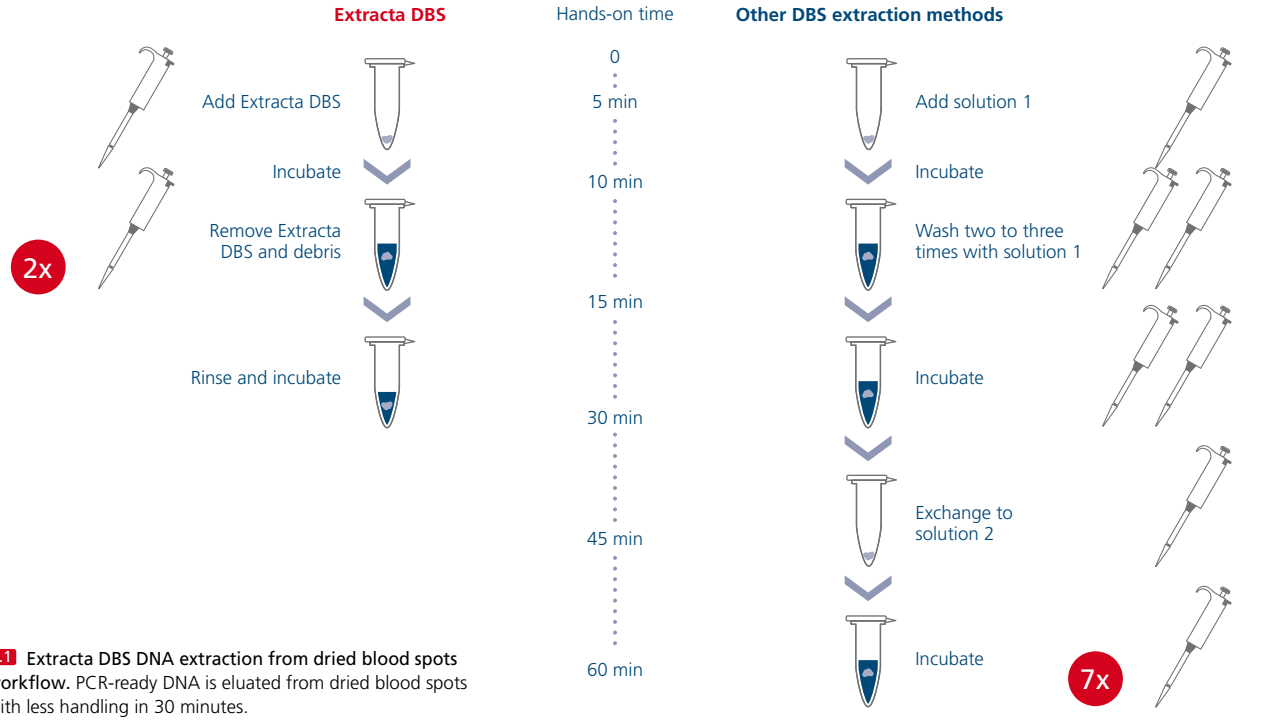
FEATURES AND BENEFITS:

- Optimized for DNA extraction from dried blood spot punches
- Single reagent for PCR-ready DNA in 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 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.

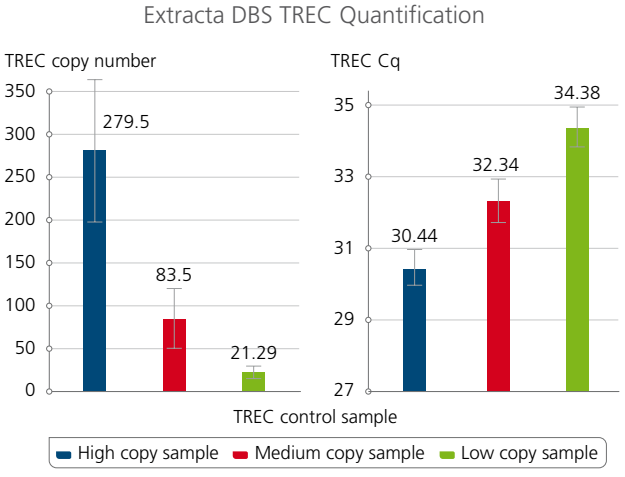
Fast, single solution extraction workflow in 30 min



2.1 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

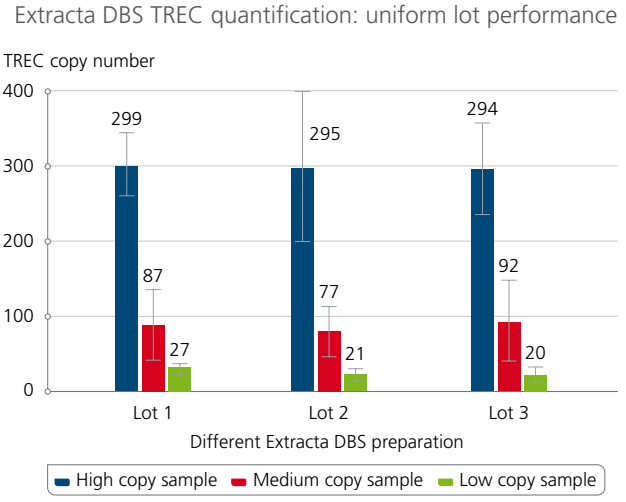
Extracta DBS is the perfect match with Quantabio ToughMix for sensitive and precise target quantification. The crude extraction combined with ToughMix, a Quantabio master mix that is tolerant to common PCR inhibitors, results in higher DNA yields independent of DNA sample inputs and qualities to enable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



2.2 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.

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.



2.3 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

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

Phase Lock Gel

Simplifies organic extraction of nucleic acid 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:

- Eliminates interphase contamination of nucleic acid solution
- 30% greater yield of nucleic acids over conventional method
- Gel barrier allows easy sample decanting
- Reduced contact with hazardous organic solvents

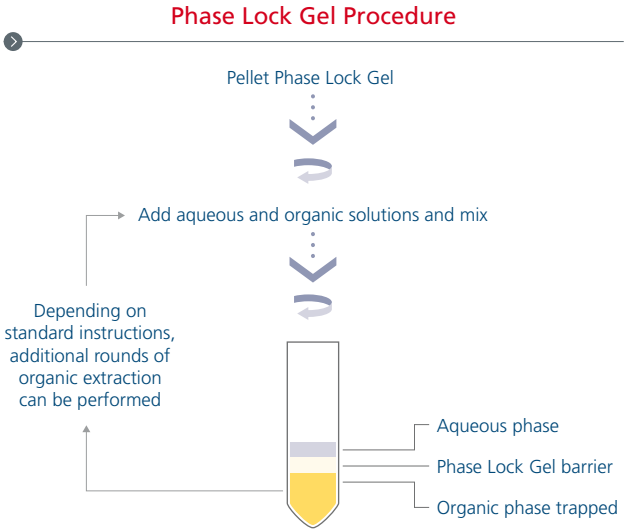
DESCRIPTION:

Phase Lock Gel (PLG) is a unique product that eliminates interphase-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 3.0

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 3.1

PRODUCT OVERVIEW

	qScript cDNA SuperMix	qScript XLT cDNA SuperMix	qScript cDNA Synthesis Kit	qScript Flex cDNA Synthesis Kit
Kit Format	Single Tube	Single Tube	Two Tubes	Five Tubes
RT Enzyme	MMLV, RNase H+	MMLV, reduced RNase H activity	MMLV, RNase H+	MMLV, RNase H+
Priming Method	Oligo(dT) & random primers	Oligo(dT) & random primers	Oligo(dT) & random primers	Oligo(dT), random primers or gene specific primer
RNA Input Range	10 pg – 1 µg	1 pg – 2 µg	10 pg – 1 µg	10 pg – 1 µg
Amplicon Length	1 kb or less	1 kb or less	1 kb or less	12 kb or less
Optimal Reaction Time	40 min	30 – 70 min	40 min	60 – 90 min
Yield (Sensitivity)	+++	++++	++	++

qScript cDNA SuperMix/qScript XLT cDNA SuperMix

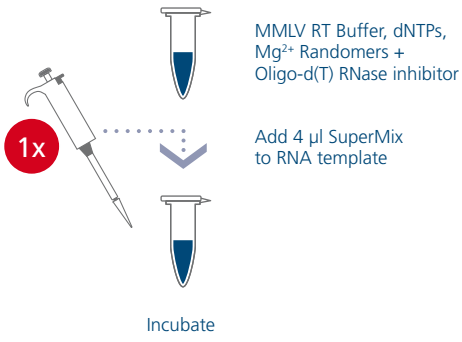
Superior cDNA synthesis in a single step

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

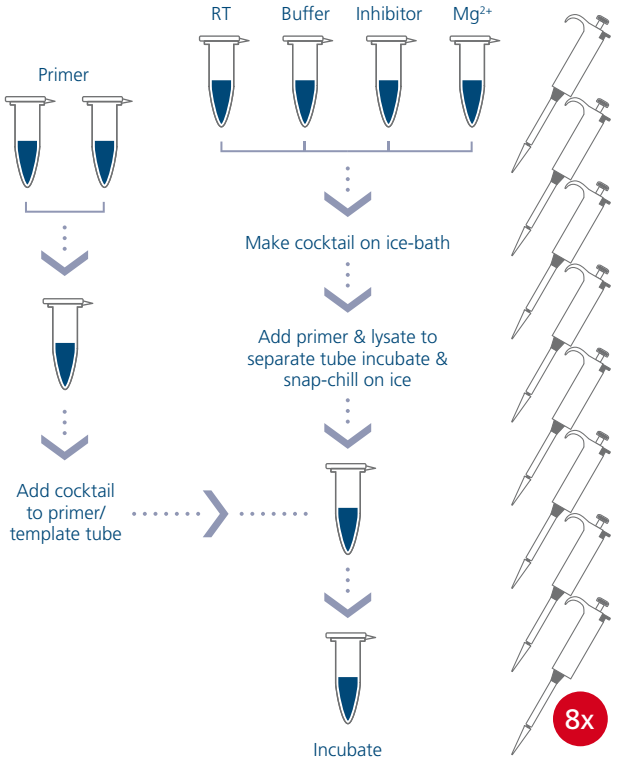
qScript cDNA SuperMix

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



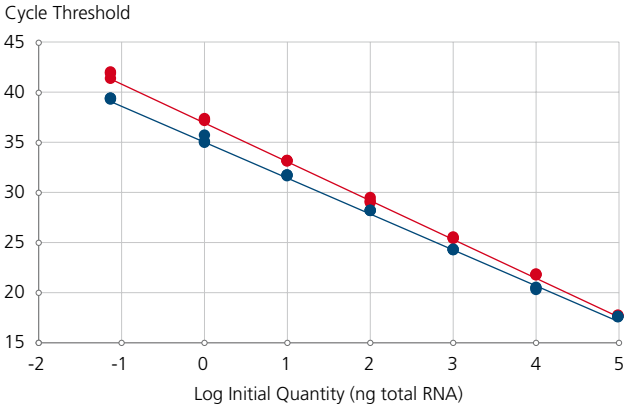
- 1 Tube
- 1 Pipetting Step
- 40 Minutes (70 min for qScript XLT Supermix)

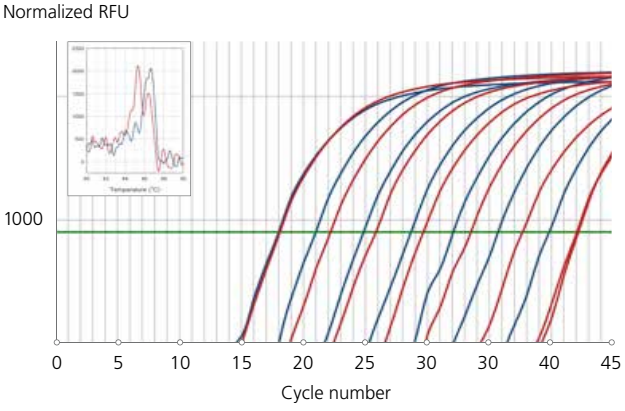
Other cDNA Kits



3.1 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

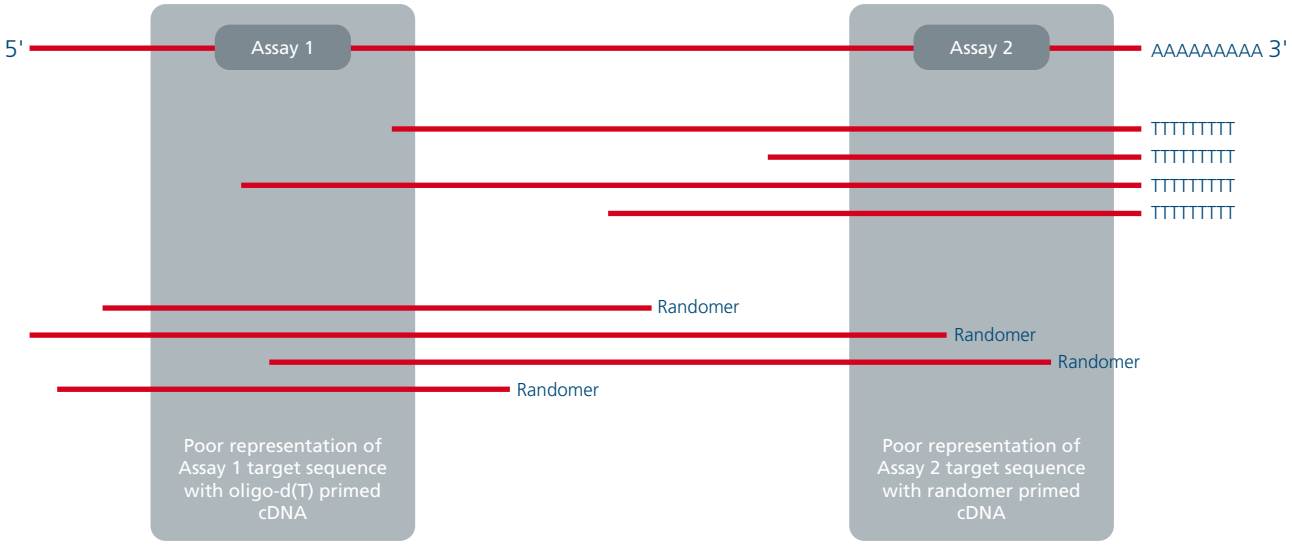




	Slope	Corr.	PCR Eff.	Sensitivity
qScript cDNA SuperMix	-3.625	0.998	88.7%	50 fg
Competitor S	-3.941	0.999	79.4%	500 fg

3.2 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

4.0 PCR

4.1 DNA Amplification

PRODUCT OVERVIEW

	Standard PCR		High Fidelity PCR	Tough PCR	HiFi & Tough PCR	Long PCR
	AccuStart II PCR SuperMix	AccuStart II GelTrack PCR SuperMix	sparQ HiFi PCR Master Mix	AccuStart II PCR ToughMix	repliQa HiFi ToughMix	AccuStart Long Range SuperMix
Features						
Concentration	2x	2x	2x	2x	**2x**	**4x**
Amplicon size	Up to 4 kb	Up to 4 kb	Up to 4 kb	Up to 4 kb	Up to 24 kb	Up to 24 kb
Extension time*	60 sec/kb	60 sec/kb	30 sec/kb	60 sec/kb	1–10 sec/kb	30–60 sec/kb
Multiplex PCR	–	–	–	–	–	Up to 6 Targets
Fidelity vs. Taq	1x	1x	80x Taq	1x	90x Taq	10–12x Taq
dU Tolerant	No	No	No	No	Yes	Yes
5' → 3' exo	•	•	N/A	•	N/A	N/A
3' → 5' exo	N/A	N/A	•	N/A	•	•
Resulting ends	3' dA overhang	3' dA overhang	Blunt	3' dA overhang	Blunt	Blunt/T overhangs
Applications						
Routine PCR	•	•	–	•	•	•
High Fidelity	–	–	•	–	**	•
Crude PCR	–	–	–	•	**	–
Fast PCR	–	–	–	–	**	–
Long PCR	–	–	–	–	•	**
High Yield	•	•	–	–	**	•
NGS Library Amplification	–	–	–	–	•	–
Available Formats						
Hot Start	•	•	•	•	•	•
Master Mix	•	•	•	•	•	•
Loading Dye Available	–	•	–	•	–	–
Packaging (rxns/units)	100 rxns, 500 rxns, 4000 rxns	100 rxns, 500 rxns, 4000 rxns	50 rxns, 250 rxns	100 rxns, 800 rxns, 4000 rxns	25 rxns, 100 rxns, 500 rxns	25 rxns, 100 rxns

* Range based on amplicon size.
** Recommended product.

repliQa HiFi ToughMix

Superior speed and inhibitor tolerance for DNA amplification requiring high fidelity

FEATURES AND BENEFITS:

- Fidelity of >90x wild type Taq
- 2–3x faster PCR results with extension rates as fast as 1 kb/sec*
- Tough Tested – tolerant to a wide range of PCR inhibitors
- Superior yield and sensitivity
- Amplification of +24 kb gDNA and +40 kb λ DNA

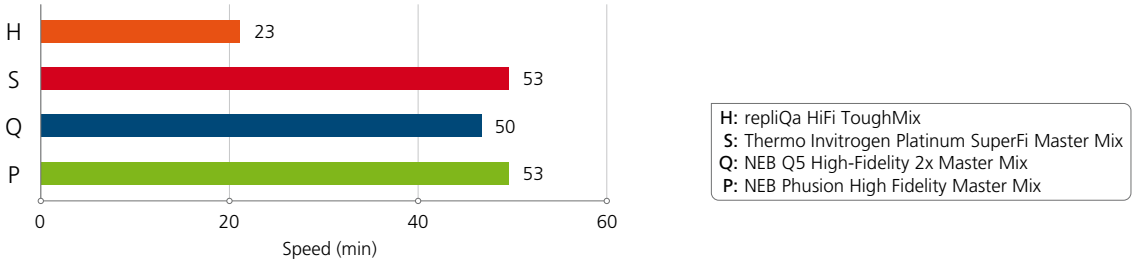
DESCRIPTION:

The repliQa HiFi ToughMix is a 2x, ready-to-use solution that contains all the components for high fidelity PCR amplification, including a genetically modified DNA polymerase coupled with hot start antibodies.

This unique, next generation master mix provides >90x higher fidelity compared to Taq, while reducing time to PCR results by 2–3x. The extreme speed is enabled by extension times as fast as 1–10 kb/sec depending on target length. The enzyme is coupled with the industry leading ToughMix which is tolerant to a wide variety of inhibitors making it suitable for routing PCR, cloning, amplicon sequencing and site directed mutagenesis.

Extreme Speed: 2–3x faster results

repliQa HiFi ToughMix has very fast extension times, ranging from 1–10 kb/sec depending on the fragment size, which can significantly shorten the time to result.

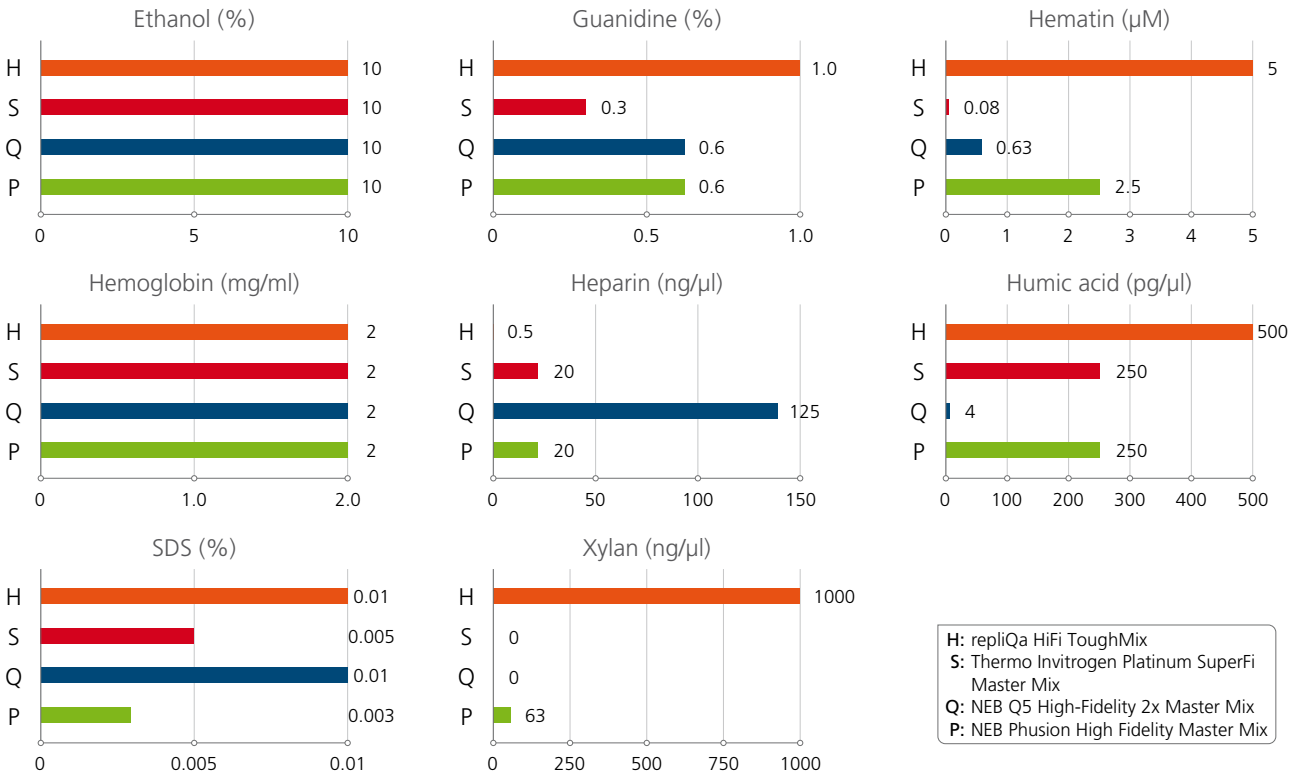


4.1 **Comparison of speed.** A 2 kb fragment was amplified in 50 µl reaction volumes according to the recommended protocol. Following a 30 s activation at 98°C; 30 cycles of PCR were performed: 98°C, 10 s; 60°C, 10 s; 68°C, 5–30 s. The thermal cycler had a ramp rate of 5°C/s.

* For fragments less than 1 kb in size.

Tough Tested: Tolerant to a wide range of PCR inhibitors

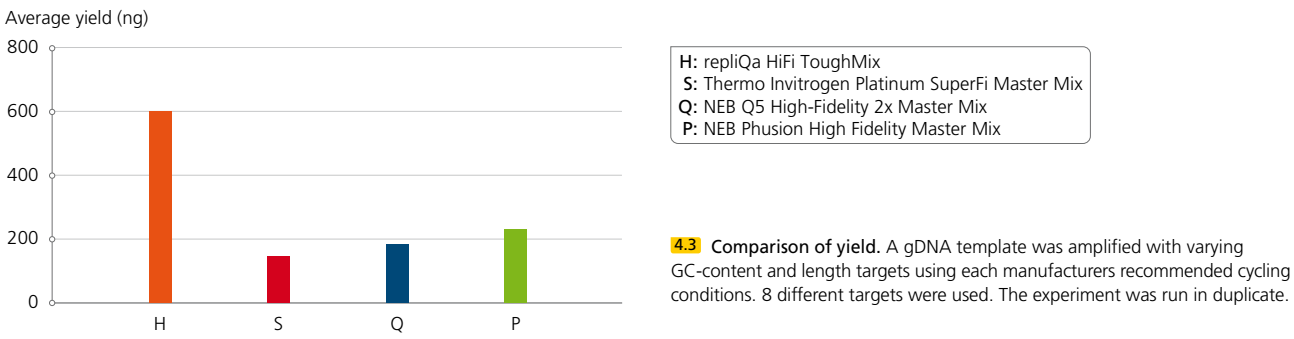
repliQa HiFi ToughMix is able to tolerate a wide range of common PCR inhibitors, allowing for amplification of crude or difficult PCR sample types.



4.2 Strong Inhibitor Resistance. A 2 kb λ DNA template was amplified using each manufacturers recommended cycling conditions with different amounts of inhibitors. The experiment was run in duplicate.

Superior Yield and Sensitivity

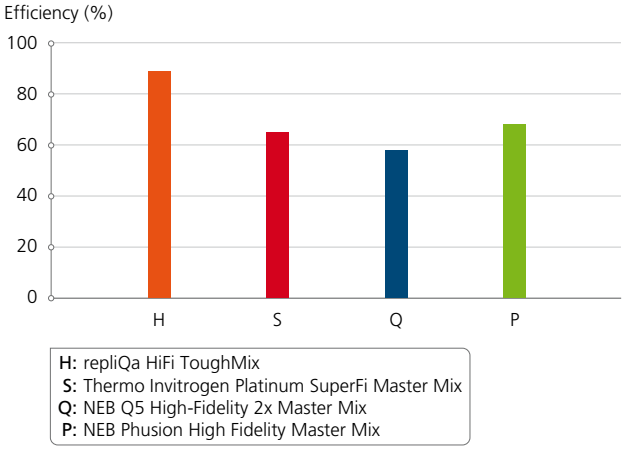
repliQa HiFi ToughMix provides higher yield and sensitivity, highlighting the enzyme efficiency. Coupled with extreme amplification speed allows PCR products to be amplified earlier and detected and lower levels.



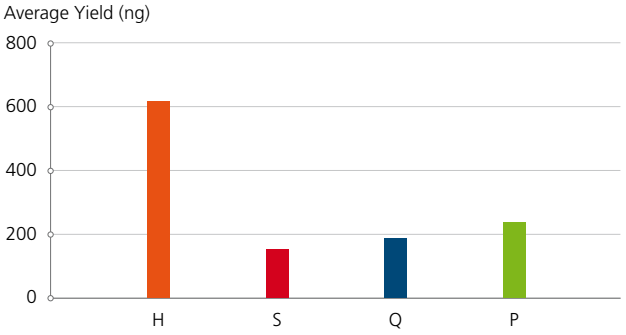
4.3 Comparison of yield. A gDNA template was amplified with varying GC-content and length targets using each manufacturers recommended cycling conditions. 8 different targets were used. The experiment was run in duplicate.

Superior Yield and Sensitivity

repliQa HiFi ToughMix demonstrates greater efficiency, at almost 90%, enabling higher yields and ultimately better sensitivity.



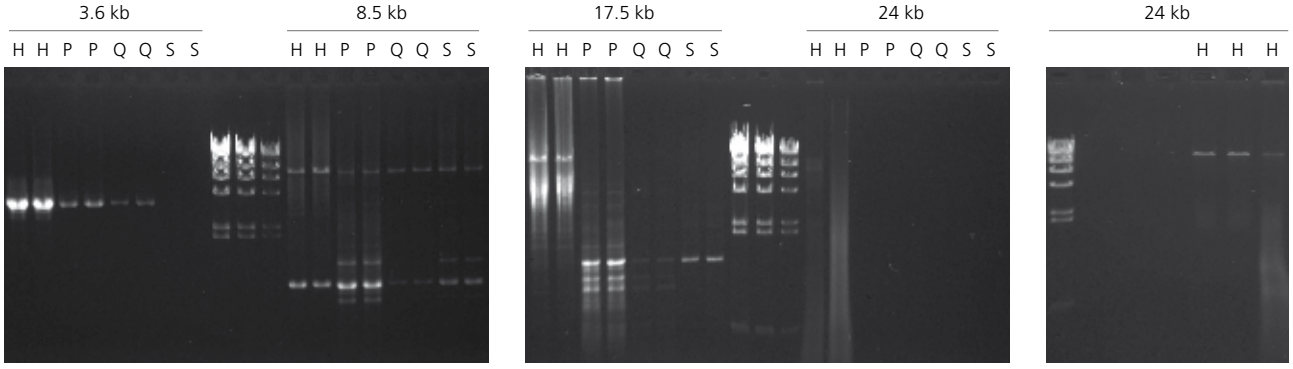
4.4 Comparison of efficiency. Amplify human genomic DNA template with varying GC-content and length targets using each master mix's recommended cycling conditions. 8 different targets were used. Ran in duplicate. See GC-content slide for more detail.



4.5 Comparison of yield. A gDNA template was amplified with varying GC-content and length targets using each manufacturers recommended cycling conditions. 8 different targets were used. The experiment was run in duplicate.

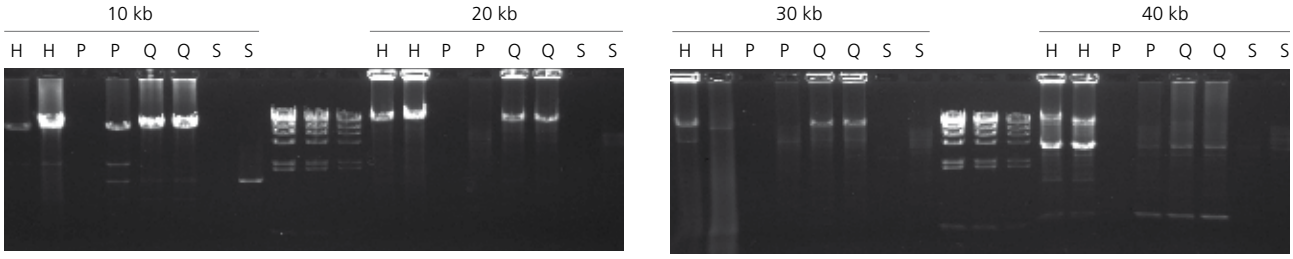
Long Amplification

repliQa HiFi ToughMix has the ability to amplify long fragments +24 kb gDNA and +40 kb λ DNA, further proving the versatility of this enzyme.



H: repliQa HiFi ToughMix **P:** NEB Phusion High Fidelity Master Mix **Q:** NEB Q5 High-Fidelity 2x Master Mix **S:** Thermo Invitrogen Platinum SuperFi Master Mix

4.6 Long Range capabilities (gDNA). A range of 3.6 kb, 8.5 kb, 17.5 kb, and 24 kb gDNA templates were amplified with varying GC-content and lengths using each manufacturers recommended cycling conditions. The experiment was run in duplicate.

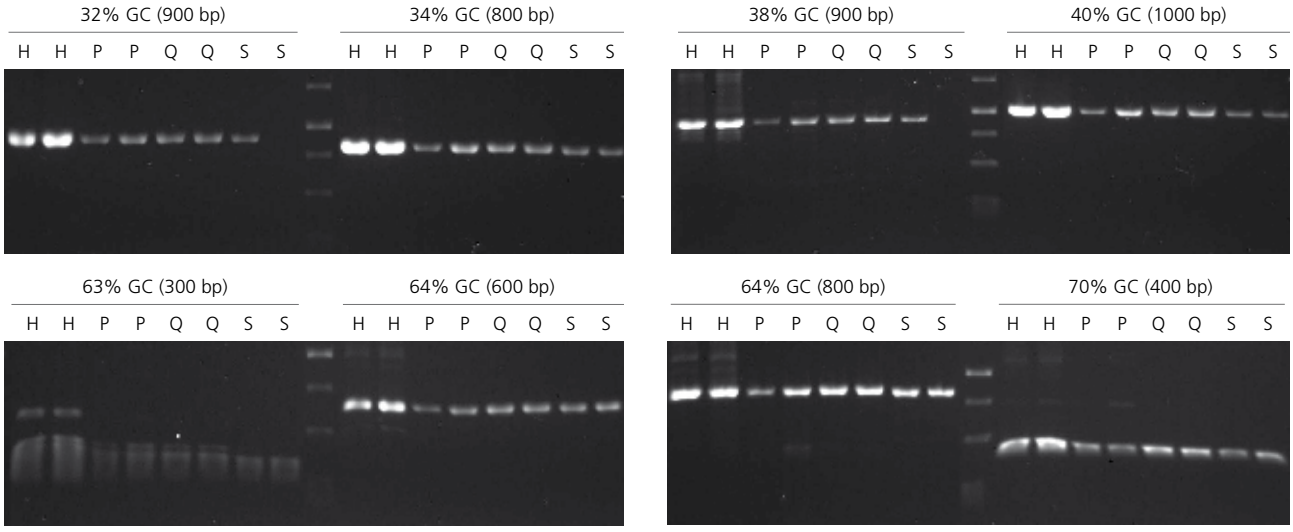


4.7 **Long Range capabilities (λDNA).** A range of 10 kb, 20 kb, 30 kb, and 40 kb λDNA templates were amplified with varying GC-content and lengths using each manufacturers recommended cycling conditions. The experiment was run in duplicate.

H: repliQa HiFi ToughMix
P: NEB Phusion High Fidelity Master Mix
Q: NEB Q5 High-Fidelity 2x Master Mix
S: Thermo Invitrogen Platinum SuperFi Master Mix

Consistent GC Tolerance

repliQa HiFi ToughMix is able to amplify varying levels of GC-content targets (32%–70% GC-rich), further enabling superior PCR performance.



4.8 **Wide GC-content tolerance range.** gDNA templates were amplified with varying GC-content and lengths using each manufacturers recommended cycling conditions. 8 different targets were used. The GC-content varied with 32%/900 base pairs (bp), 34%/800 bp, 38%/900 bp, 40%/1000 bp, 63%/300 bp, 64%/600 bp, 64%/800 bp and 70%/400 bp. The experiment was run in duplicate.

H: repliQa HiFi ToughMix
P: NEB Phusion High Fidelity Master Mix
Q: NEB Q5 High-Fidelity 2x Master Mix
S: Thermo Invitrogen Platinum SuperFi Master Mix

ORDER INFO

Product Name	Quantabio Catalog Number	Size
repliQa HiFi ToughMix - 25	95200-025	25 rxns
repliQa HiFi ToughMix - 100	95200-100	100 rxns
repliQa HiFi ToughMix - 500	95200-500	500 rxns

AccuStart Long Range SuperMix

Superior sensitivity and multiplexing for DNA amplification of long targets

FEATURES AND BENEFITS:

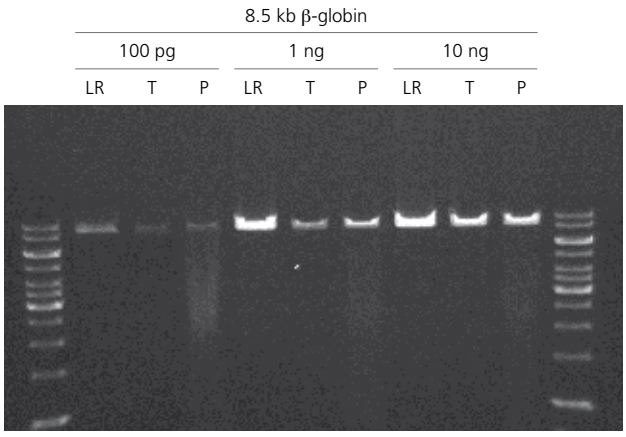
- Amplify +24 kb gDNA and +40 kb lambda DNA
- 4x concentration enables superior sensitivity with low inputs (100 pg)
- Multiplex up to 6 targets with each target up to 6 kb in length
- Stabilized, single-tube SuperMix minimizes pipetting errors and hands-on-time

DESCRIPTION:

The AccuStart Long Range SuperMix is a 4x solution that contains all the components for long range target amplifications, including a blend of two hot-start thermostable DNA polymerases and an optimized buffer. This SuperMix enables routine and easy amplification with high accuracy (>10x Taq) and accommodates targets with broad GC-content (no separate GC buffer needed). This product is also capable of multiplexing and is suitable for End Point PCR, template prep for Sanger Sequencing, NGS, Cloning and HLA typing.

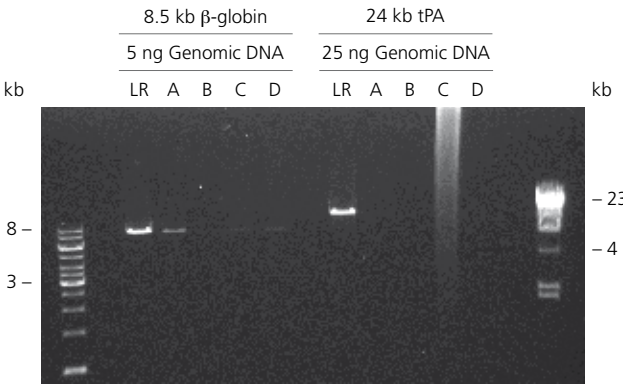
Superior Sensitivity: Improved yields across a range of DNA inputs and target sizes

The AccuStart Long Range SuperMix can amplify DNA inputs as low as 100 pg, across a wide range of target sizes.



LR AccuStart Long Range SuperMix **T** Takara LR ver2.1
P Promega GoTaq LR

4.9 Comparison of sensitivity and yield. 8.5 kb β-globin fragments were amplified in 50 µl reaction volumes according to the recommended protocol. Reaction inputs varied from 100 pg – 10 ng. Following a 3 min activation at 95°C; 30 cycles of PCR were performed: 92°C, 30 s; 65°C, 6 min; 72°C, 10 min.

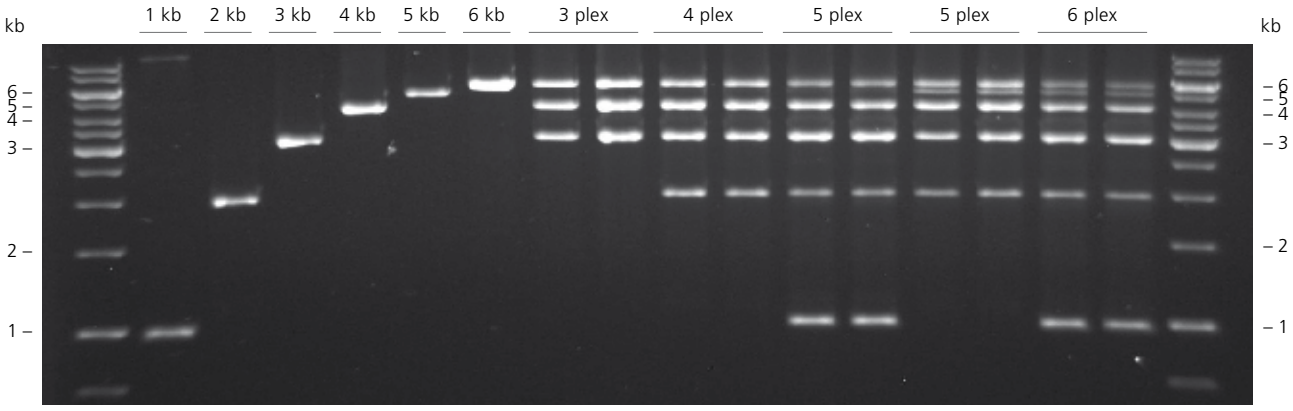


LR AccuStart Long Range SuperMix **A** NEB LA Master Mix
B KAPA ReadyMix **C** AccuPrime Taq High Fidelity
D Platinum Taq High Fidelity

4.10 Comparison of yield over fragment length. 8.5 kb β-globin and 24 kb tPA fragments were amplified in 50 µl reaction volumes according to the recommended protocol. Reaction inputs were 5 ng and 25 ng for the 8 kb and 24 kb fragments, respectively. Following a 3 min activation at 95°C; 27 cycles of PCR were performed: 92°C, 30 s; 65°C, 6 min (8.5 kb), 12 min (24 kb); 72°C, 10 min.

Maximized Multiplexing: Amplify 6 targets up to 6 kb each

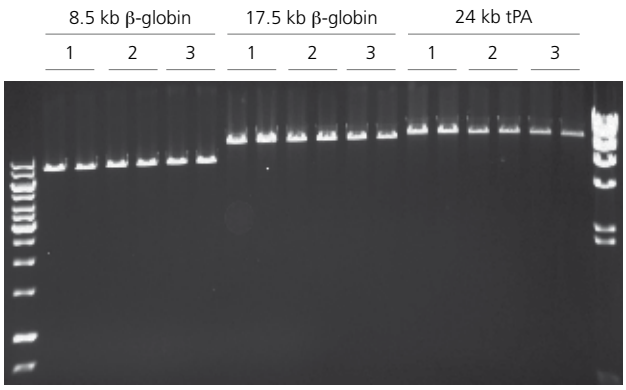
The AccuStart Long Range SuperMix can multiplex 6 targets up to 6 kb each, further speeding up experiments, reducing costs and allowing for more data to be derived per run.



4.11 Strong multiplexing capabilities. Multiplex PCR reactions were performed for amplification of 2 kb, 3 kb, 4 kb and 5 kb BRCA 1 targets with 1 kb and 6 kb BRCA 2 targets from 10 ng human genomic DNA template. Reactions were run on MJ Research PTC-200 Thermal Cycler. Following a 2 min activation of 95°C; 27 cycles of PCR were performed: 92°C, 30 s, 65°C, 8 min; 72°C, 10 min. 4 µl of PCR products were analyzed on a 0.7% agarose gel.

Reliable lot-to-lot reproducibility

AccuStart Long Range SuperMix is manufactured in a state of the art facility under an ISO13485 quality system which provides consistent lot-to-lot reproducibility.



1 Lot 1
2 Lot 2
3 Lot 3

4.12 Lot-to-Lot consistency. 8.5 kb β-globin, 17.5 kb and 24 kb tPA fragments were amplified in 50 µl reaction volumes according to the recommended protocol. Reaction inputs were 5 ng, 10 ng and 25 ng for the 8 kb, 17.5 kb and 24 kb fragments, respectively. Following a 3 min activation at 95°C; 27 cycles of PCR were performed: 92°C, 30 s; 65°C, 6 min (8.5 kb), 8 min (17.5 kb), 12 min (24 kb); 72°C, 10 min. 5 µl of PCR products were analyzed on a 0.5% agarose gel with a DNA marker.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
AccuStart Long Range SuperMix - 25 R	95199-025	25 rxns
AccuStart Long Range SuperMix - 100 R	95199-100	100 rxns

sparQ HiFi PCR Master Mix

High-fidelity library amplification while maintaining even coverage

FEATURES AND BENEFITS:

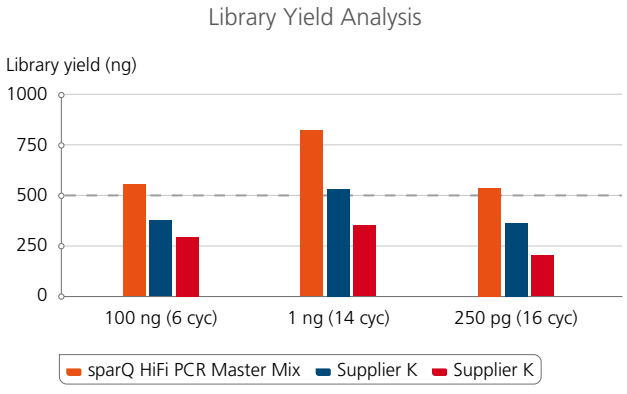
- HiFi DNA polymerase engineered to minimize amplification bias
- Increased amplification efficiency resulting in higher yields
- Uniform coverage across challenging AT- and GC-rich regions
- Robust amplification from input DNA as low as 250 pg

DESCRIPTION:

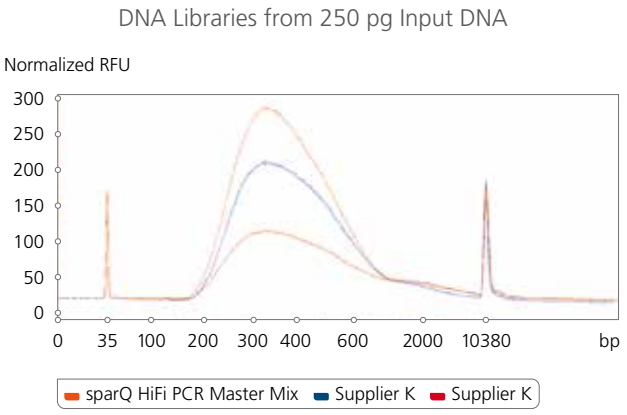
The sparQ HiFi PCR Master Mix is a high-fidelity, high-efficiency PCR master mix for NGS workflows requiring DNA library amplification prior to sequencing. The included primer mix allows amplification of DNA libraries flanked by adapters containing the P5 and P7 sequences required for Illumina® sequencing platforms. The hot-start, proofreading DNA polymerase used in the sparQ HiFi PCR Mater Mix is specifically engineered to improve library amplification efficiency while reducing PCR-derived artifacts, resulting in higher library yields and better coverage uniformity. This kit supports low DNA input from 250 pg and efficient amplification of AT- and GC-rich regions with minimal bias.

Higher library amplification efficiency

Specially designed for sensitive, high efficiency library amplification from a broad range of DNA input, the sparQ HiFi PCR Master Mix minimizes the number of amplification cycles needed to achieve the threshold required for sequencing. The result is >45% higher library yields while reducing PCR-derived artifacts.



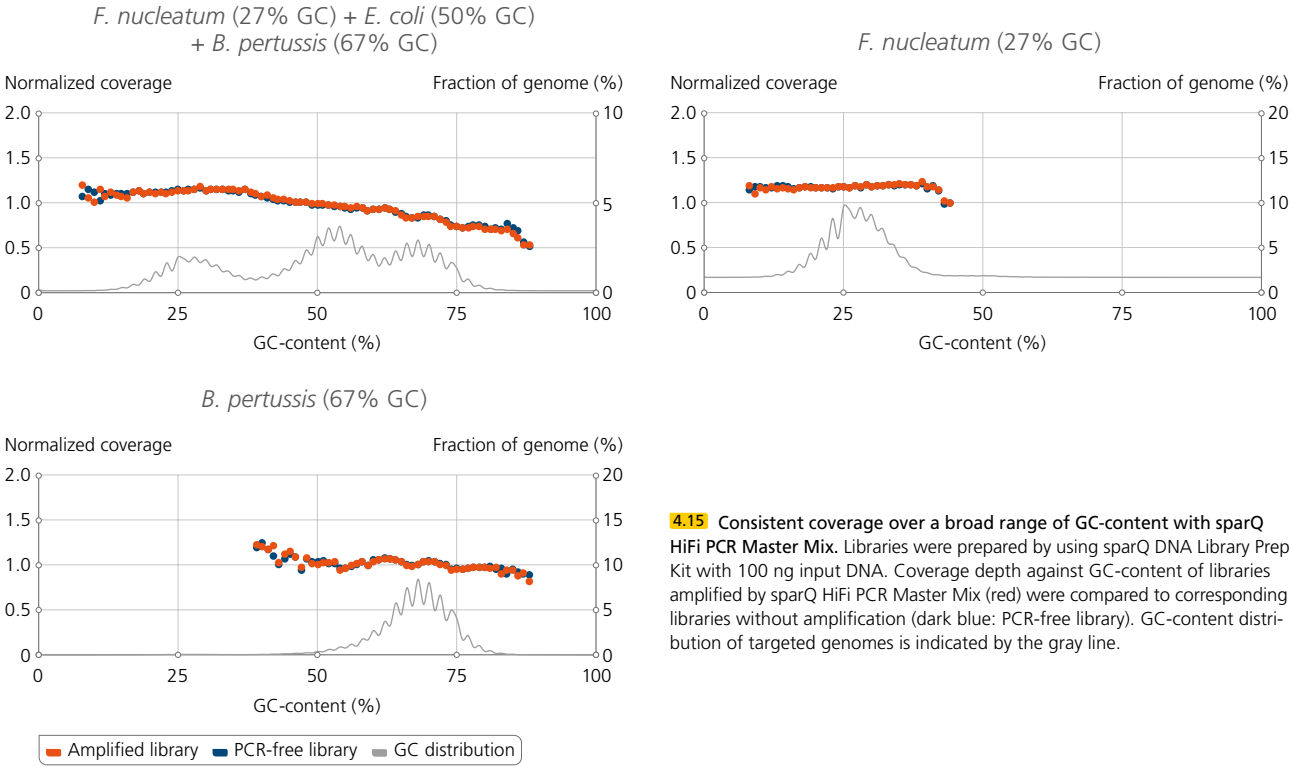
4.13 Library amplification with sparQ HiFi PCR Master Mix resulted in higher yields. Libraries were prepared from Covaris-sheared human genomic DNA with sparQ DNA library prep kit prior to library amplification. Pre-amplified libraries were then amplified using sparQ HiFi PCR Master Mix (orange) or equivalent kit from Supplier K (blue) and Supplier N (red) with identical PCR 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 method and qPCR-based quantification method (data not shown).



4.14 sparQ HiFi PCR Master Mix demonstrates high efficiency library amplification from low input. 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 the Agilent BioAnalyzer. Libraries were amplified using sparQ HiFi PCR Master Mix (orange) or equivalent kit from Supplier K (blue) and Supplier N (red) with identical amplification cycle numbers (16 cycles for 250 pg input DNA).

Superior coverage uniformity

Libraries amplified by sparQ HiFi PCR Master Mix provide uniform coverage across a broad range of GC-content, similar to corresponding libraries without PCR. Even coverage ensures greater sequencing depth or multiplexing capabilities.



4.15 Consistent coverage over a broad range of GC-content with sparQ HiFi PCR Master Mix. 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 the 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)
Related Products		
sparQ DNA Frag & Library Prep Kit - 24 R	95194-024	24 rxns
sparQ DNA Frag & Library Prep Kit - 96 R	95194-096	96 rxns
sparQ DNA Library Prep Kit - 24 R	95191-024	24 rxns
sparQ DNA Library Prep Kit - 96 R	95191-096	96 rxns
sparQ PureMag Beads - 5 ml	95196-005	5 ml
sparQ PureMag Beads - 60 ml	95196-060	60 ml
sparQ PureMag Beads - 450 ml	95196-450	450 ml
sparQ Universal Library Quant Kit - 100 R	95210-100	100 rxns
sparQ Universal Library Quant Kit - 500 R	95210-500	500 rxns

AccuStart II GelTrack PCR SuperMix

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 PCR ToughMix

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

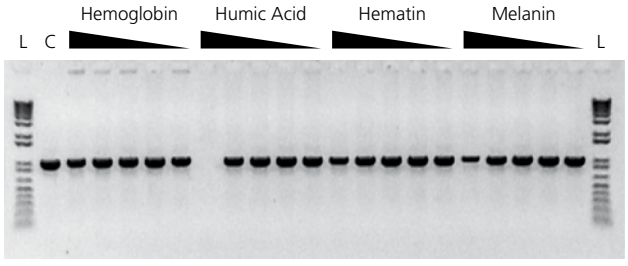
FEATURES AND BENEFITS:

- Stabilized 2x PCR SuperMix enables convenient room-temperature setup and is unaffected by repetitive freeze-thaw
- High-yielding, ultrapure modified Taq DNA polymerase delivers robust, reliable duplex assay performance
- Stringent, ultrapure antibody hotstart ensures sensitive and specific target amplification
- Separate electrophoretic mobility dye reduces risk of post-PCR cross contamination with gel electrophoresis

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.

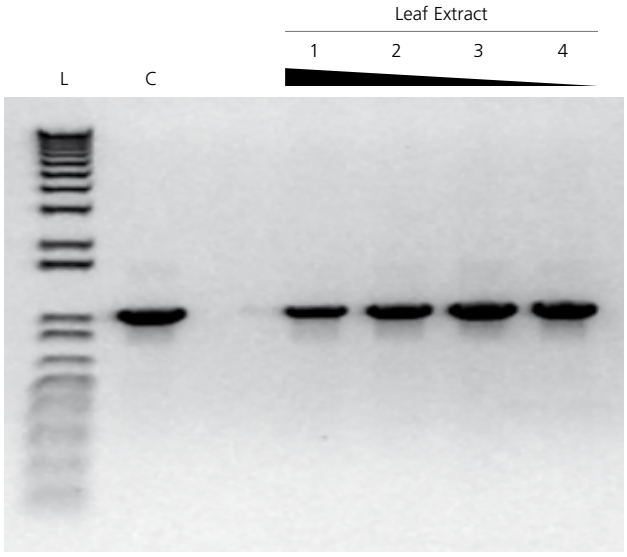
30 cycle PCR; 1 x 10^4 copies TcR DNA (1052 bp amplicon)



4.16 **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)



4.17 **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 II Taq DNA Polymerase

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)

AccuStart II PCR Genotyping Kit

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

FEATURES AND BENEFITS:

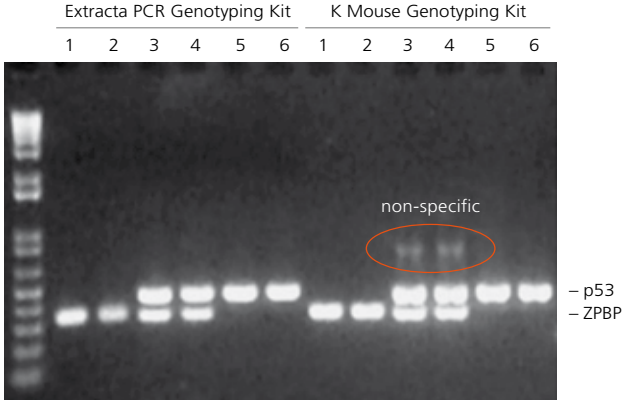
- Premixed electrophoresis mobility loading dye reduces chances for post-PCR cross contamination
- Stabilized 2x PCR SuperMix enables convenient room-temperature setup
- High-yielding, ultrapure modified Taq DNA polymerase delivers robust, reliable duplex assay performance
- Stringent, ultrapure antibody 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.

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.



4.18 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.

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

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 - 1000 µl	95062-01K	1000 µl

4.2 RNA Amplification

qScript XLT 1-Step RT-PCR Kit

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

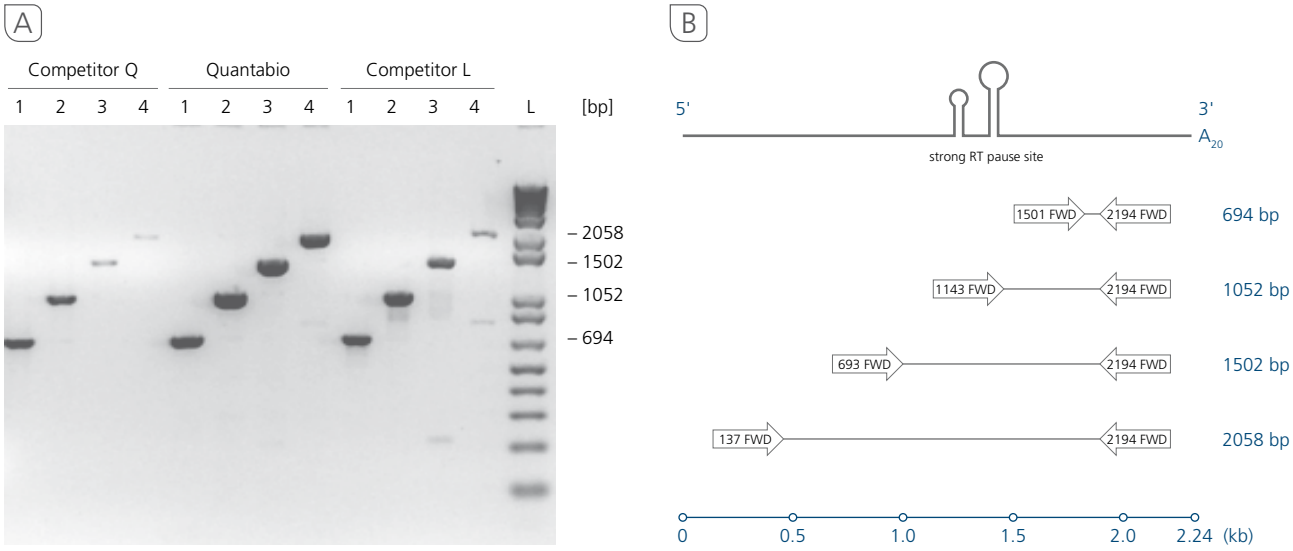
The qScript XLT 1-Step RT-PCR kit provides highly sensitive detection of large, complex RNA in challenging starting materials supporting high-fidelity downstream applications

FEATURES AND BENEFITS:

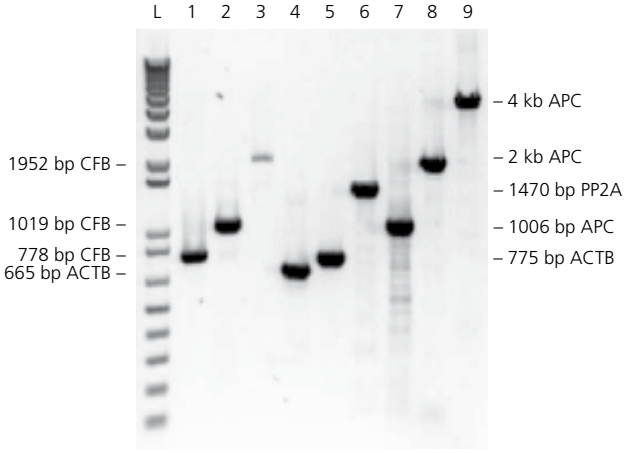
- ToughMix reagent technology withstands PCR inhibitors commonly found in plant and animal tissues, environmental sources, clinical specimens and complex food matrices
- Optional GelTrack dye streamlines workflow for gel electrophoresis
- Temperature stabilized for support reaction assembly at convenient 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 1-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.



4.19 **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 containing 0.25 mg/ml ethidium bromide.



4.20 **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 1-Step RT-PCR Kit - 20 R	95143-020	20 x 25 µl rxns
qScript XLT 1-Step RT-PCR Kit - 200 R	95143-200	200 x 25 µl rxns

5.0 Real-Time qPCR

5.1 DNA Amplification

PRODUCT OVERVIEW

	PerfeCta qPCR ToughMix	PerfeCta qPCR ToughMix, UNG	Accustart Genotyping ToughMix	PerfeCta Multiplex qPCR SuperMix
Concentration	2x	2x	2x	2x
Performance	++++	++++	++++	+++
Inhibitor Tolerance	✓	✓	✓	✓
Chemistry	Probe	Probe	Probe	Probe
Sample Type	gDNA, cDNA	gDNA, cDNA	gDNA, cDNA	gDNA, cDNA
Cycling Mode	Standard or Fast	Standard or Fast	Standard or Fast	Standard or Fast
Fast Cycling Compatibility	✓	✓	✓	✓
Multiplex Compatibility	Up to 2 targets	Up to 2 targets	Up to 2 targets	Up to 5 targets
Carryover contamination control	–	Includes heat-labile UNG and a blend of dTTP / dUTP	–	–
Application	Gene Expression	Gene Expression	SNP Genotyping	Gene Expression

	PerfeCta Multiplex qPCR ToughMix	PerfeCta FastMix II	PerfeCta SYBR Green SuperMix	PerfeCta SYBR Green FastMix
Concentration	5x	2x	2x	2x
Performance	++++	+++	++	++
Inhibitor Tolerance	✓	–	–	–
Chemistry	Probe	Probe	SYBR Green	SYBR Green
Sample Type	gDNA, cDNA	gDNA, cDNA	gDNA, cDNA	gDNA, cDNA
Cycling Mode	Standard or Fast	Standard or Fast	Standard only	Standard or Fast
Fast Cycling Compatibility	–	–	–	✓
Multiplex Compatibility	Up to 5 targets	Up to 2 targets	–	–
Carryover contamination control	–	–	–	–
Application	Gene Expression	Gene Expression	Gene Expression, microRNA Expression, ChIP Analysis	Gene Expression Analysis

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:

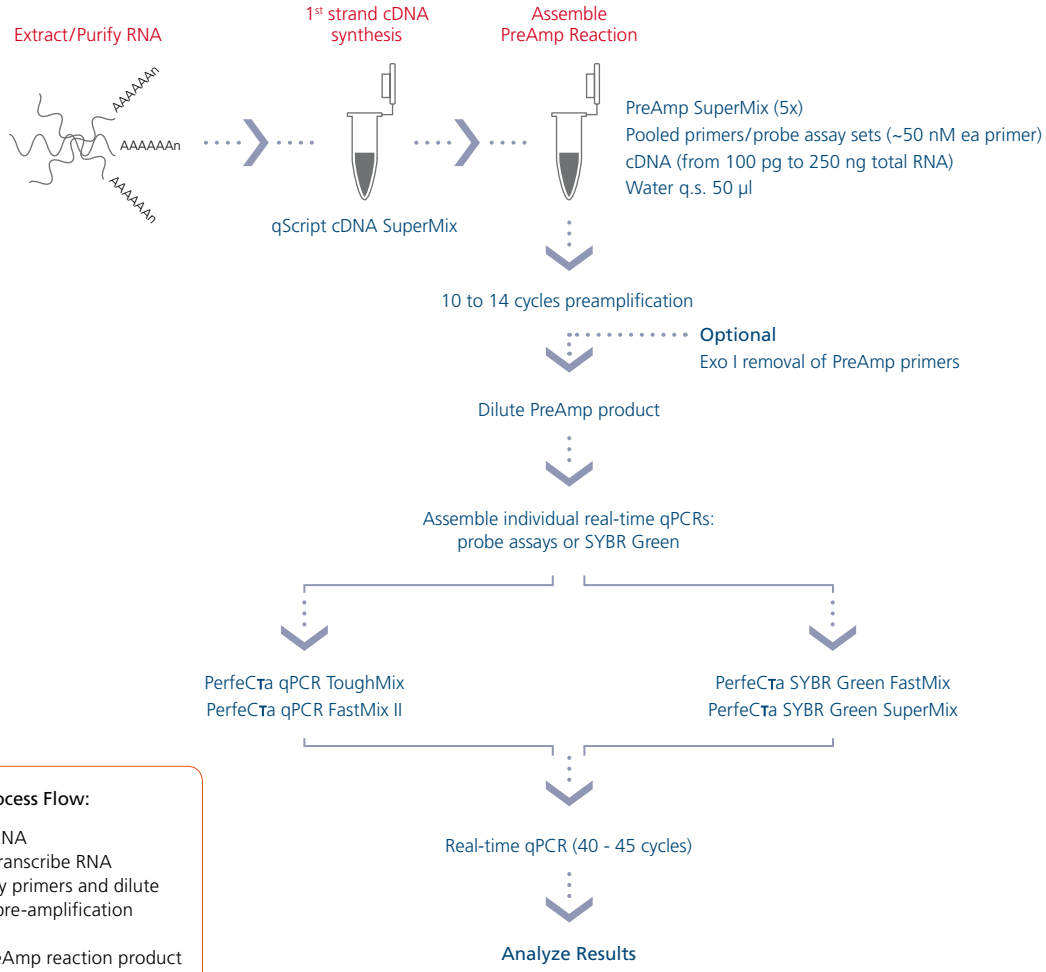
- Unbiased, linear pre-amplification of up to 100 DNA targets
- 5x concentrated SuperMix maximizes sample input volume with dilute cDNA templates
- Superior assay sensitivity and specificity with ultrapure AccuStart II enzyme technology – maximum-yielding Taq DNA polymerase mutant controlled by stringent, 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 - 40 R	95146-040	40 x 50 µl rxns

SYBR Detection

PerfeCTa SYBR Green SuperMix/FastMix

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

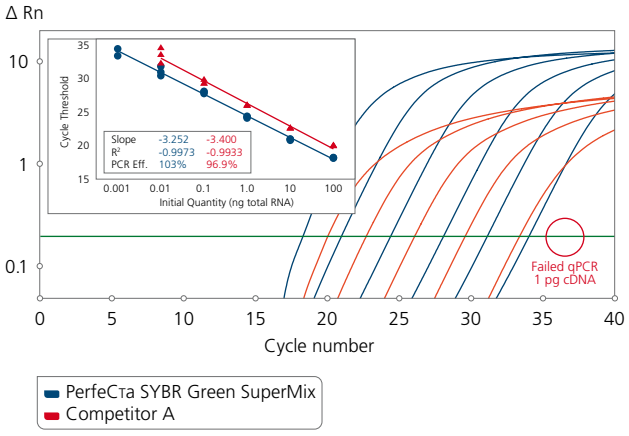
FEATURES AND BENEFITS:

- 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)

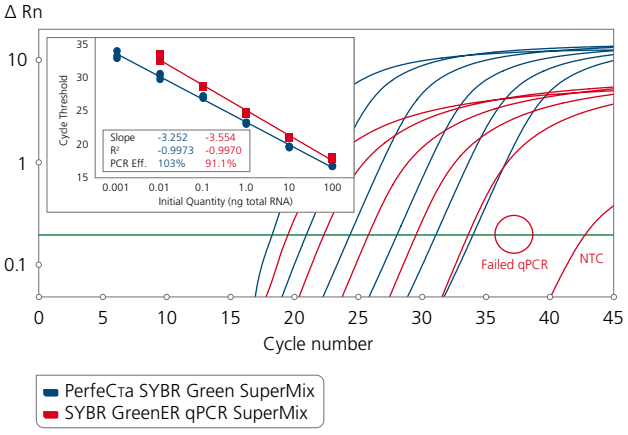
DESCRIPTION:

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.

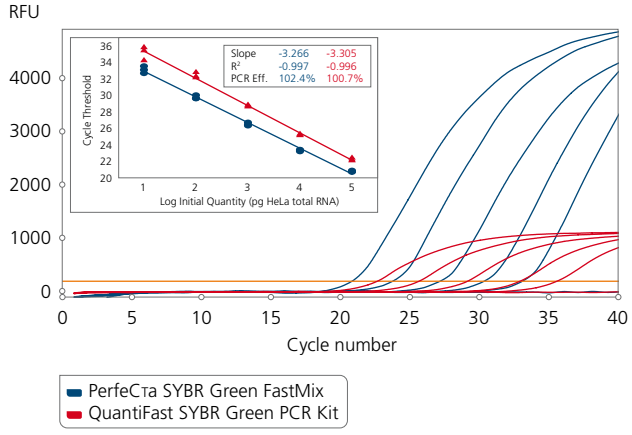
Single-tube reagents are 2x concentrated ready-to-use reaction cocktails containing all necessary components, except primers and DNA template for quantitative PCR. Proprietary formulation stabilizes SYBR Green I dye to deliver maximum efficiency, sensitivity, and robust fluorescent signal.



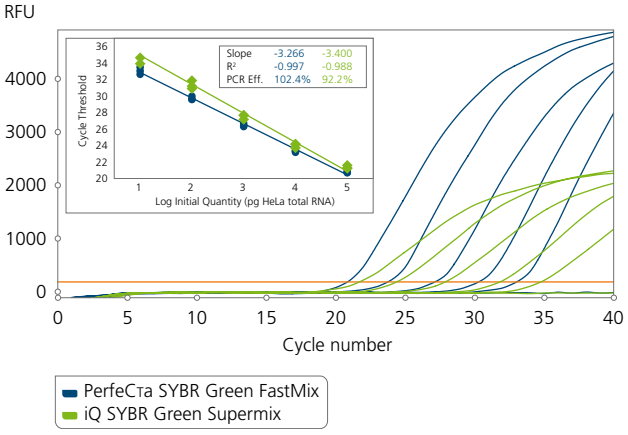
5.1 PKCA target amplified from log-fold serial dilutions of qScript synthesized cDNA from HeLa cell total RNA (100 ng – 1 pg) with either PerfeCTa SYBR Green SuperMix or Power SYBR Green PCR Master Mix.



5.2 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.



5.3 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.



5.4 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 - 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 - 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 - 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 - 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 - 1250 R	95056-500	10 x 1.25 ml
PerfeCta SYBR Green SuperMix, Low ROX - 5000 R	95056-02K	1 x 50 ml

Probe Detection

PRODUCT OVERVIEW

	Conventional 1-Step RT-PCR	Tough-Tested MultiPlex 1-Step RT-qPCR	Tough-Tested 1-Step Probe-based RT-qPCR	1-Step Probe-based RT-qPCR	1-Step SYBR-based RT-qPCR
Kit	qScript XLT 1-Step RT-PCR Kit	UltraPlex 1-Step ToughMix	qScript XLT 1-Step RT-qPCR ToughMix	qScript 1-Step RT-qPCR Kit	qScript 1-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

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

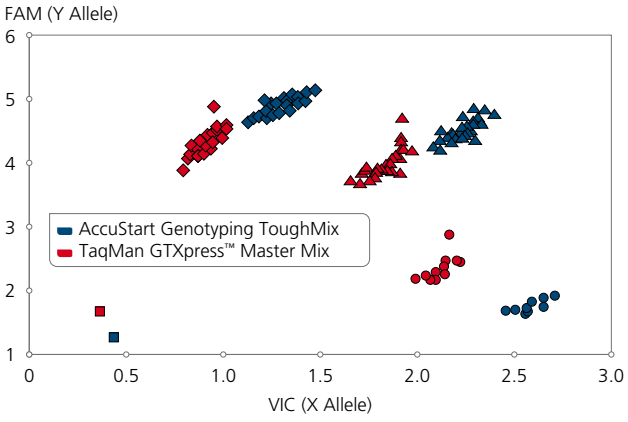
FEATURES AND BENEFITS:

- 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:

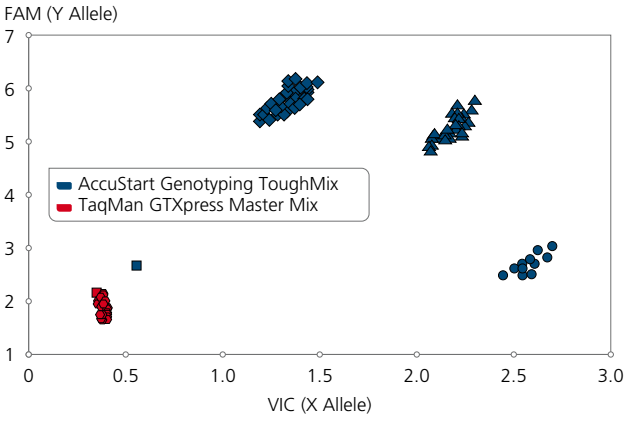
Genotyping ToughMix is a 1-tube qPCR SuperMix reagent compatible with all dual-label (hydrolysis) probe chemistries for both fast and conventional PCR cycling protocols or instruments. This proprietary formulation has been rigorously optimized to destabilize single base-pair mismatches to ensure precise 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



5.5 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



5.6 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, 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 - 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)

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

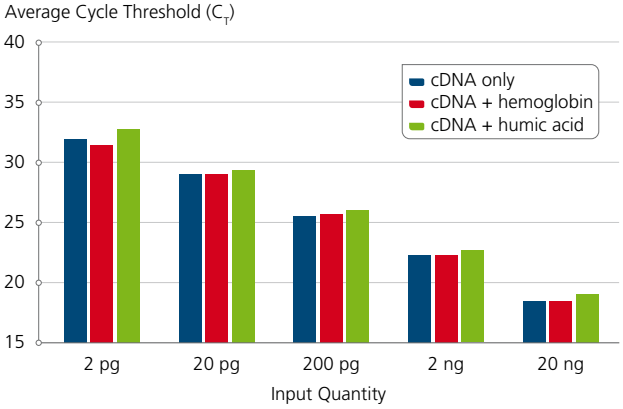
DESCRIPTION:

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 and 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	–	✓

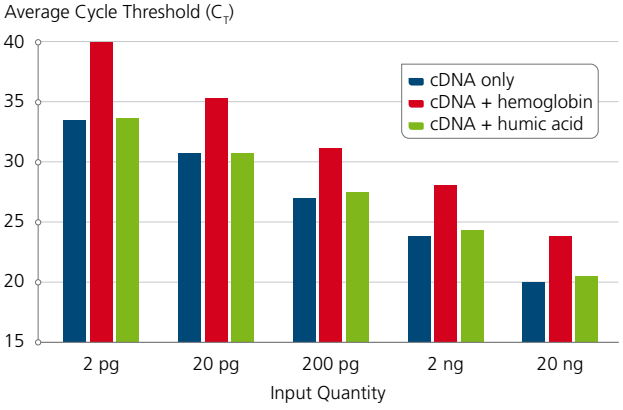
Effect of PCR Inhibitors on qPCR of MYC cDNA

PerfeCTa qPCR ToughMix



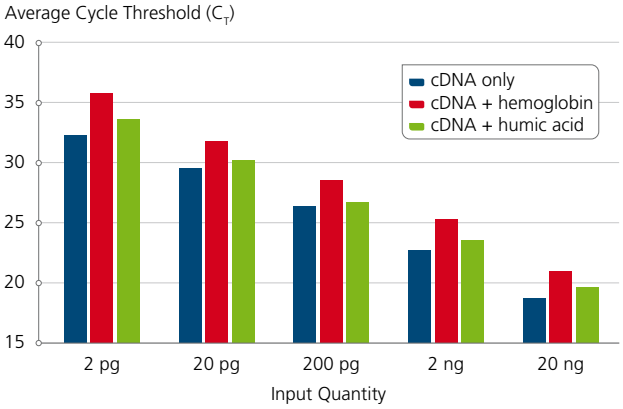
Slope	-3.365	-3.267	-3.420
R^2	-0.9989	-0.9970	-0.9988
Int.	27.57	27.42	28.08
PCR Eff.	98.2%	102.4%	96.1%

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%

5.7 **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 - 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 - 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 - 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 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

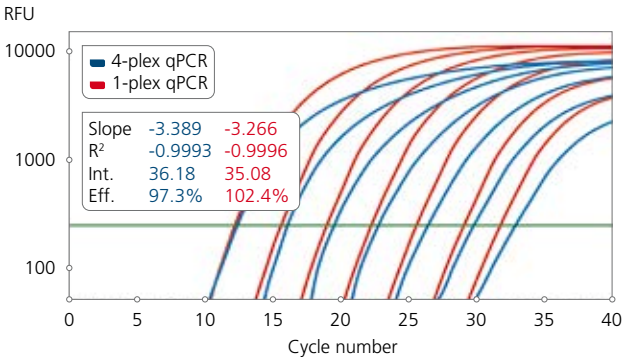
FEATURES AND BENEFITS:

- 1-tube SuperMix minimizes pipetting steps, simplifying reaction assembly and improving accuracy
- 5x concentrated reagent provides greater sensitivity and more flexibility with dilute DNA samples
- 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, plants, soil, and 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 MultiPlex qPCR ToughMix 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.



5.8 **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 broad, 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 MultiPlex qPCR SuperMix 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 - 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 - 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

5.2 RNA Amplification

PRODUCT OVERVIEW

	Quantitative RT-qPCR					
	Qscript lyo 1-step	Script XLT 1-Step RT-qPCR ToughMix	qScript 1-Step SYBR Green RT-qPCR	qScript 1-Step RT-qPCR Kit	UltraPlex 1-Step ToughMix	qScript 1-Step Virus ToughMix
Features						
Kit Format	1 Tube	1 Tube	2 Tubes	2 Tubes	1 Tube	1 Tube
RT Enzyme	MMLV, reduced RNase activity	MMLV, reduced RNase activity	MMLV, RNase H+	MMLV, RNase H+	MMLV, reduced RNase activity	MMLV, reduced RNase activity
Concentration	N/A	2x	2x	2x	2x	2x
Yield	++++	++++	+++	+++	++++	++++
Total RNA Input Range	0.5 pg – 500 pg	1 pg – 100 ng	1 pg – 100 ng	1 pg – 1 µg	1 pg – 100 ng	1 pg – 100 ng
Amplicon Length	70 – 300 bp	70 – 300 bp	0 – 200 bp	70 – 300 bp	70 – 300 bp	70 – 300 bp
Hot Start	N/A	N/A	N/A	N/A	Yes	Yes
Applications						
Inhibitor Tolerant	–	•	–	–	•	•
High Yield	–	•	–	–	•	•
Multiplex PCR	•	•	–	–	•	•
Available Formats						
Master Mix	•	•	–	–	•	•
Lyophilized	•	–	–	–	–	–
Packaging (rxns/units)	24 rxns	100 rxns, 500 rxns, 2000 rxns	100 rxns, 400 rxns	50 rxns, 200 rxns	100 rxns, 500 rxns, 1000 rxns	100 rxns, 500 rxns, 2000 rxns

UltraPlex 1-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 1-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 1-Step ToughMix- 100 R	95166-100	100 x 20 µl rxns
UltraPlex 1-Step ToughMix - 500 R	95166-500	500 x 20 µl rxns
UltraPlex 1-Step ToughMix - 1000 R	95166-01K	1000 x 20 µl rxns
UltraPlex 1-Step ToughMix ROX - 100 R	95167-100	100 x 20 µl rxns
UltraPlex 1-Step ToughMix ROX - 500 R	95167-500	500 x 20 µl rxns
UltraPlex 1-Step ToughMix Low ROX - 100 R	95168-100	100 x 20 µl rxns
UltraPlex 1-Step ToughMix Low ROX - 500 R	95168-500	500 x 20 µl rxns
UltraPlex 1-Step ToughMix Low ROX - 1000 R	95168-01K	1000 x 20 µl rxns

qScript 1-Step Virus ToughMix

Superior sensitivity for viral RNA detection

FEATURES AND BENEFITS:

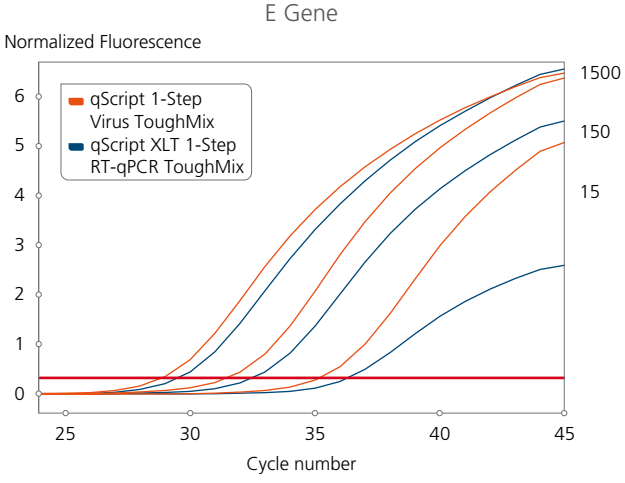
- Superior Sensitivity – higher amplification yields enable lower copy detection
- Tough Tested – tolerant to a wide range of PCR inhibitors
- Multiplexing – highly sensitive detection for up to four targets

DESCRIPTION:

qScript 1-Step Virus ToughMix is a 2x, ready-to-use, master mix for rapid detection of RNA viruses such as Flu-A, Flu-B, and SARS-CoV-2, using one-step, or single-tube reverse transcription quantitative PCR (RT-qPCR). The mix has been optimized for maximum sensitivity to enable reliable quantification of very low input quantities of RNA using dual-labeled hydrolysis probe detection chemistries such as TaqMan® probes in single or multiplexed assay formats. qScript 1-Step Virus ToughMix contains all required components for RT-qPCR except RNA template and primer/probe assays.

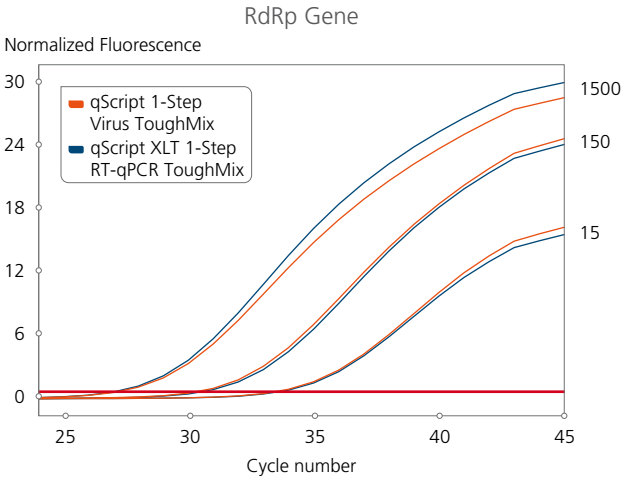
Higher Yields, Better Sensitivity:

qScript 1-step Virus ToughMix was benchmarked against the gold standard qScript XLT 1-Step RT-qPCR ToughMix for detection of varying amounts of SARS-CoV-2 RNA in a background of human total RNA. Multiplexed RT-qPCR of the E-gene (FAM), RdRp (HEX) and RNase P Internal Control (ROX) was carried out on the Q qPCR cycler: 10 min at 50°C; 1 min at 95°C; followed by 45 cycles of 5 sec at 95°C; 10 sec at 60°C. Both products generated excellent results. However, the qScript 1-Step Virus ToughMix demonstrated earlier detection and robust amplification curves (higher signal amplitude) for low copy E-gene detection.



5.9 E Gene comparing qScript 1-Step Virus ToughMix vs. qScript XLT 1-Step RT-qPCR ToughMix. 15, 150 and 1500 copies of RNA were compared. qScript 1-Step Virus ToughMix improves low copy RT-qPCR performance at both 150 and 15 copies of RNA.

Quantabio recommends both of these high performing products for RNA virus detection. While highly similar in composition, the improved specificity provided by the reverse transcription hot-start of the qScript 1-Step Virus ToughMix often translates to improved sensitivity and fewer false negative calls at or near the limit of detection (LOD), particularly in multiplexed assays.



5.10 RdRp Gene comparing qScript 1-Step Virus ToughMix vs. qScript XLT 1-Step RT-qPCR ToughMix. 15, 150 and 1500 copies of RNA were compared. qScript 1-Step Virus ToughMix improves low copy RT-qPCR performance.

Features	qScript 1-Step Virus ToughMix	qScript XLT 1-Step RT-qPCR ToughMix
Formulation	2X Master Mix	2X Master Mix
Taq Hot Start	Yes	Yes
RT Hot Start	Yes	No
Chemistry	1 tube SuperMix	1 tube SuperMix
Performance	++++	++++
Cycling Conditions	Fast or Standard qPCR Cycling	Fast or Standard qPCR Cycling
Multiplex Capability	Up to 4 targets	Up to 4 targets
Sensitivity	++++	+++
Inhibitor Tolerance	Yes	Yes

ORDER INFO

Product Name	Quantabio Catalog Number	Size
qScript 1-Step Virus ToughMix - 100 R	95131-100	100 x 20 µl rxns
qScript 1-Step Virus ToughMix - 500 R	95131-500	500 x 20 µl rxns
qScript 1-Step Virus ToughMix - 2000 R	95131-02K	2000 x 20 µl rxns
qScript 1-Step Virus ToughMix Low ROX - 100 R	95212-100	100 x 20 µl rxns
qScript 1-Step Virus ToughMix Low ROX - 500 R	95212-500	500 x 20 µl rxns
qScript 1-Step Virus ToughMix Low ROX - 2000 R	95212-02K	2000 x 20 µl rxns

qScript XLT 1-Step RT-qPCR ToughMix

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

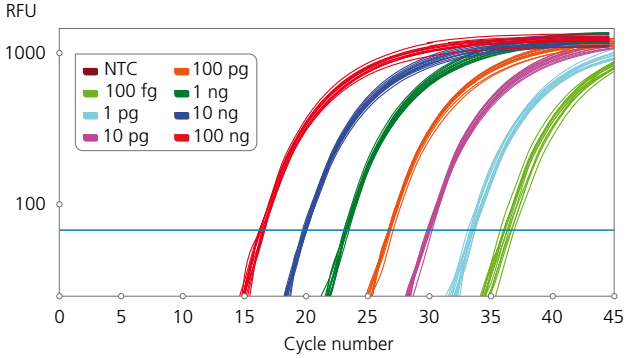
FEATURES AND BENEFITS:

- 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 both fast and standard thermal cycling conditions

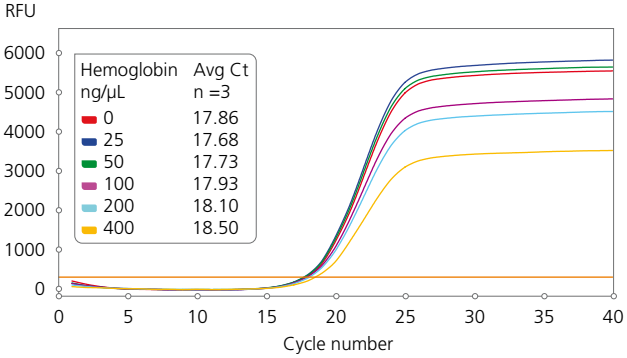
DESCRIPTION:

qScript XLT 1-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.



5.11 Broad linear dynamic range, low Limit of Detection.



5.12 Enables performance in the presence of inhibitors.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
qScript XLT 1-Step RT-qPCR ToughMix - 100 R	95132-100	100 x 20 µl rxns
qScript XLT 1-Step RT-qPCR ToughMix - 500 R	95132-500	500 x 20 µl rxns
qScript XLT 1-Step RT-qPCR ToughMix - 2000 R	95132-02K	2000 x 20 µl rxns
qScript XLT 1-Step RT-qPCR ToughMix, ROX - 100 R	95133-100	100 x 20 µl rxns
qScript XLT 1-Step RT-qPCR ToughMix, ROX - 500 R	95133-500	500 x 20 µl rxns
qScript XLT 1-Step RT-qPCR ToughMix, ROX - 2000 R	95133-02K	2000 x 20 µl rxns
qScript XLT 1-Step RT-qPCR ToughMix, Low ROX - 100 R	95134-100	100 x 20 µl rxns
qScript XLT 1-Step RT-qPCR ToughMix, Low ROX - 500 R	95134-500	500 x 20 µl rxns

qScript 1-Step SYBR Green RT-qPCR

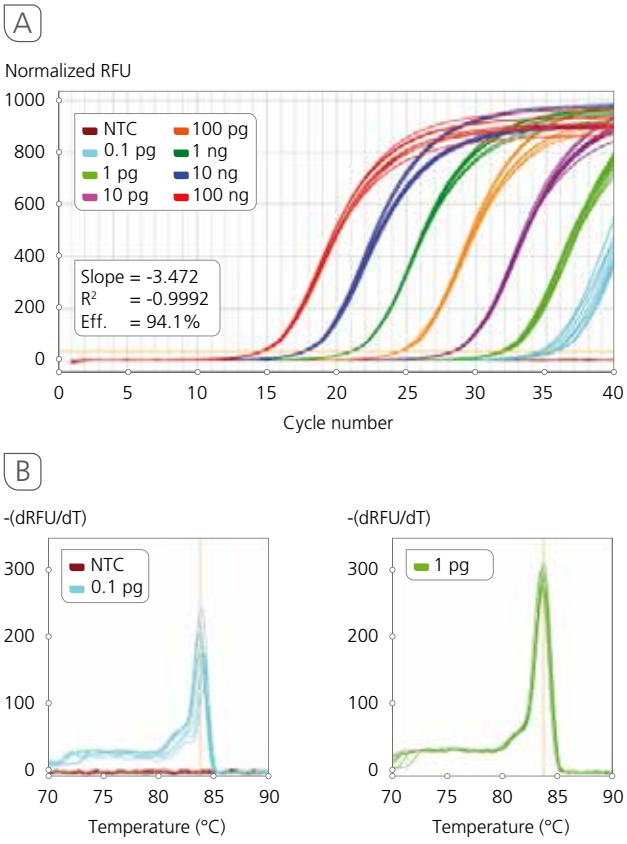
Sensitive, dye-based RNA quantification with gene specific primers in a single, seamless reaction mixture without opening the tube prior to PCR

FEATURES AND BENEFITS:

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

DESCRIPTION:

The qScript 1-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.



5.13 1-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 1-Step SYBR Green RT-qPCR Kit and 200 nM each GAPD specific primers (PrimerBank ID 7669492a2, Wang, X. and Seed, (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 1-Step SYBR Green RT-qPCR Kit - 200 R	95087-200	200 x 50 µl rxns
qScript 1-Step SYBR Green RT-qPCR Kit, Low ROX - 200 R	95089-200	200 x 50 µl rxns

qScript 1-Step RT-qPCR Kit

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 1-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 1-Step RT-qPCR Kit - 200 R	95057-200	200 x 50 µl rxns
qScript 1-Step RT-qPCR Kit, ROX - 200 R	95058-200	200 x 50 µl rxns
qScript 1-Step RT-qPCR Kit, Low ROX - 200 R	95059-200	200 x 50 µl rxns

Qscript lyo 1-step

Dry. Stable. Easy. Better. One-step RT-qPCR

FEATURES AND BENEFITS:

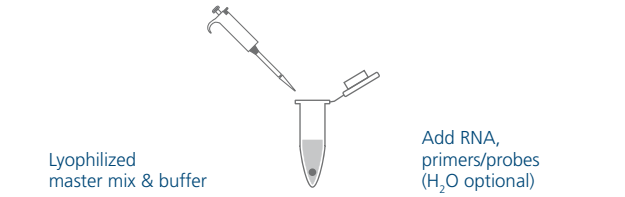
- **Lyophilized Single Tube Format** – Easy-to-use, reduces cross-contamination
- **High Sensitivity & Specificity** – Detect as low as 0.5 pg RNA
- **Wide Dynamic Range** – 0.5 – 500 pg RNA
- **Superior Multiplexing** – Plex up to 5 targets per reaction
- **Eliminate Freezer Storage** – up to 9 months stability at room temperature

DESCRIPTION:

Qscript lyo 1-step is a lyophilized single-reaction reagent optimized for highly sensitive and reproducible one-step RT-qPCR using hydrolysis probes. The reagent contains a hot-start thermo-stable polymerase, a genetically engineered reverse transcriptase as well as other components to ensure higher performance detection of up to 5 targets with maximum sensitivity and specificity. The enhanced stability of the freeze-dried master mix enables convenient shipping and storage at room temperature. The single tube reaction facilitates easy reaction set up while preventing potential cross-contamination.

5.14 Qscript lyo 1-step lyospheres.

Convenient setup

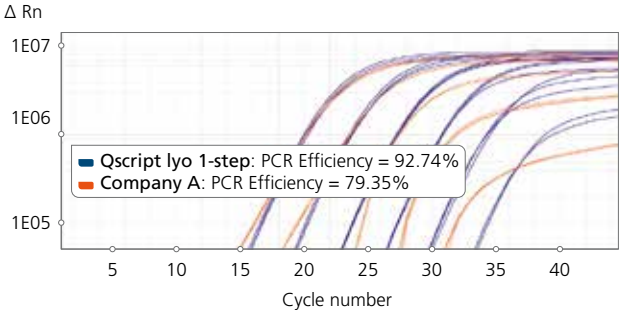


Single tube

No master mix/buffer transfer. Never cross-contaminate.

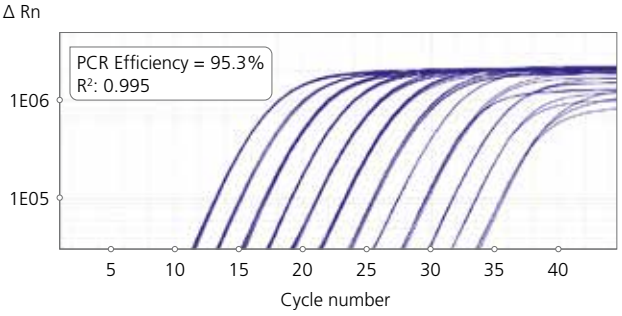
5.15 Just add RNA, primers and probes.

Better sensitivity enables greater detection



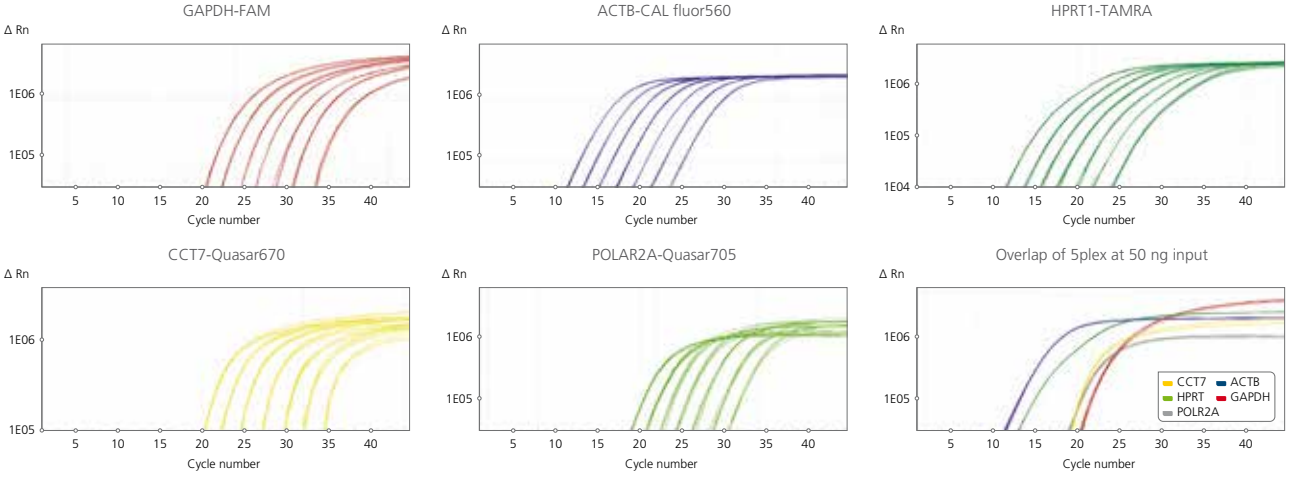
5.16 Qscript lyo 1-step demonstrates higher sensitivity and fluorescence signal than a market-leading one-step RT-qPCR product (liquid). The figure shows a LDHA assay with 10-fold serial diluted universal human RNA (50 ng to 0.5 pg) as template. 20 µl of LDHA primer/probe was used to resuspend each lyosphere, followed by addition of 5 µl of RNA target. RT-qPCR was carried out on a QuantStudio 7 instrument with the following conditions: 50°C 15 min, 95°C 3 min, 40 cycles: 95°C 15 sec, 60°C 60 sec.

High performance across a range of inputs



5.17 Qscript lyo 1-step was tested with 12 serial dilutions of Human total RNA (4x from 1250 ng to 0.3 pg). 20 µl of LDHA primer/probe was used to resuspend each lyosphere, followed by addition of 5 µl of RNA target. RT-qPCR was carried out on a QuantStudio 7 instrument with the following conditions: 50°C 15 min, 95°C 3 min, 40 cycles: 95°C 15 sec, 60°C 60 sec.

Multiplex up to 5 targets per assay



5.18 Qscript lyo 1-step was tested with a 5-plex assay with 7 serial dilutions of Human total RNA (4x from 50 ng to 30 pg). 20 µl of primers/probes was used to resuspend each lyosphere, followed by addition of 5 µl of RNA target. RT-qPCR was carried out on a QuantStudio 7 instrument with the following conditions: 50°C 15 min, 95°C 3 min, 40 cycles: 95°C 15 sec, 60°C 60 sec.

Ask about custom sizes and capabilities

ORDER INFO

Product Name	Quantabio Catalog Number	Size
Qscript lyo 1-step - 24 R	95198-024	24 rxns

sparQ DNA Library Prep Kit

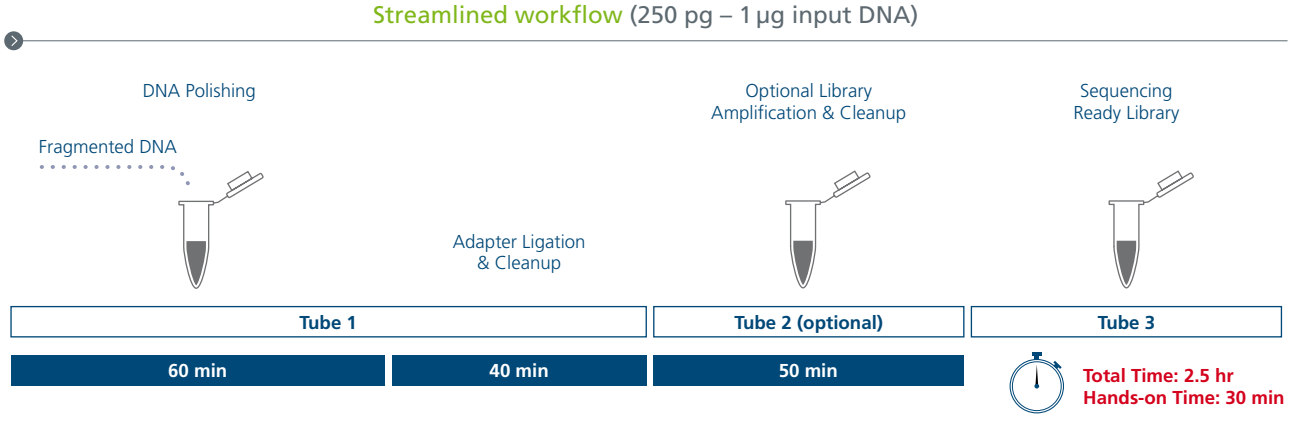
Streamlined, versatile single-tube solution for high quality library prep

FEATURES AND BENEFITS:

- Fast, easy single-tube solution completes library prep in 2.5 hours
- Suitable for a wide range of input amounts from as low as 250 pg
- Optimized chemistry ensuring superior library prep sensitivity and efficiency
- Higher library yields compared to other library prep kits
- High efficiency enables PCR-free workflow from 100 ng input

DESCRIPTION:

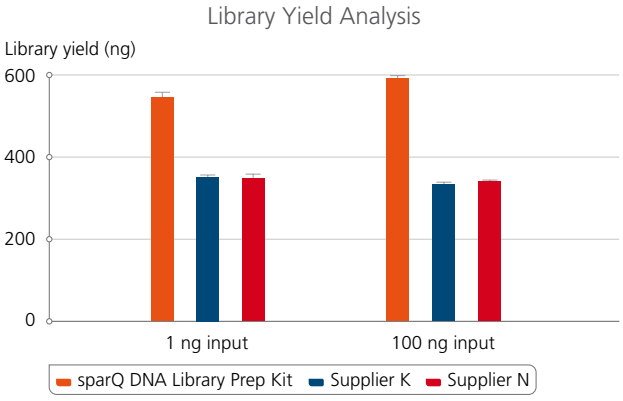
The sparQ DNA Library Prep Kit is optimized for the rapid construction of DNA libraries from fragmented double-stranded DNA for sequencing on Illumina NGS platforms. The simplified protocol speeds up library prep to 2.5 hours with minimal hands-on time and accommodates DNA input amounts from 250 pg to 1 µg. DNA polishing reactions are streamlined into a single step to convert fragmented DNA into 5'-phosphorylated and 3'-dA-tailed DNA fragments. This is followed by high efficiency adapter ligation in the same tube. PCR-free workflows are enabled from 100 ng of starting material. If library amplification is required, the HiFi PCR Master Mix and Primer Mix ensure even amplification with minimal bias. The kit is compatible with input amounts from 250 pg to 1 µg DNA and multiple sample types.



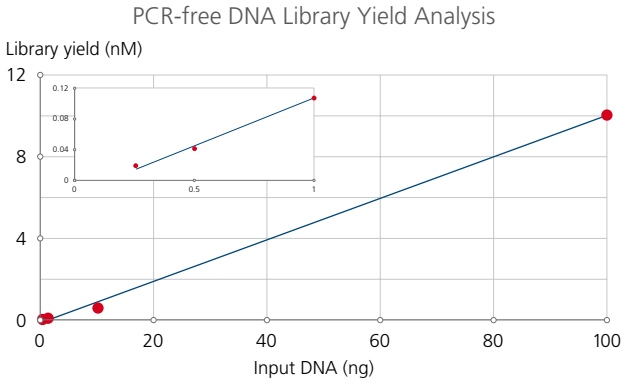
6.1 **The streamlined workflow combines DNA polishing and adapter ligation in a single tube for rapid construction of libraries from fragmented DNA.** An optional step using the HiFi PCR Master Mix and Primer Mix ensures even library amplification with minimal bias.

Higher library yields and consistent library prep efficiency

The quality of a library prep depends heavily on the efficiency and sensitivity of the enzymes involved in DNA polishing and adapter ligation steps. The sparQ enzymes have been engineered for optimal sensitivity and efficiency, supporting the construction of adapter-ligated libraries from a broad range of input DNA from as little as 250 pg. The unique proprietary cocktail of enzymes ensures exceptional library yields – 50% more than other commercial library prep kits.



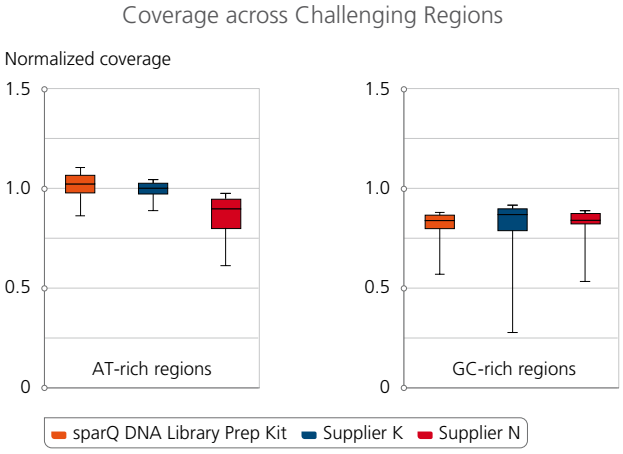
6.2 sparQ DNA Library Prep Kit produces high quality libraries from a broad range of DNA inputs with significantly higher yields. Libraries were prepared with Covaris-sheared human genomic DNA (250 bp average size) using kit manufacturers' instructions. Amplified libraries (6 PCR cycles for 100 ng input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



6.3 sparQ DNA Library prep Kit resulted in consistent library prep efficiency across a broad range of sample inputs. Libraries were prepared from Covaris-sheared human genomic DNA with sparQ DNA Library Prep Kit without library amplification. Preamplified libraries were quantified with qPCR-based method.

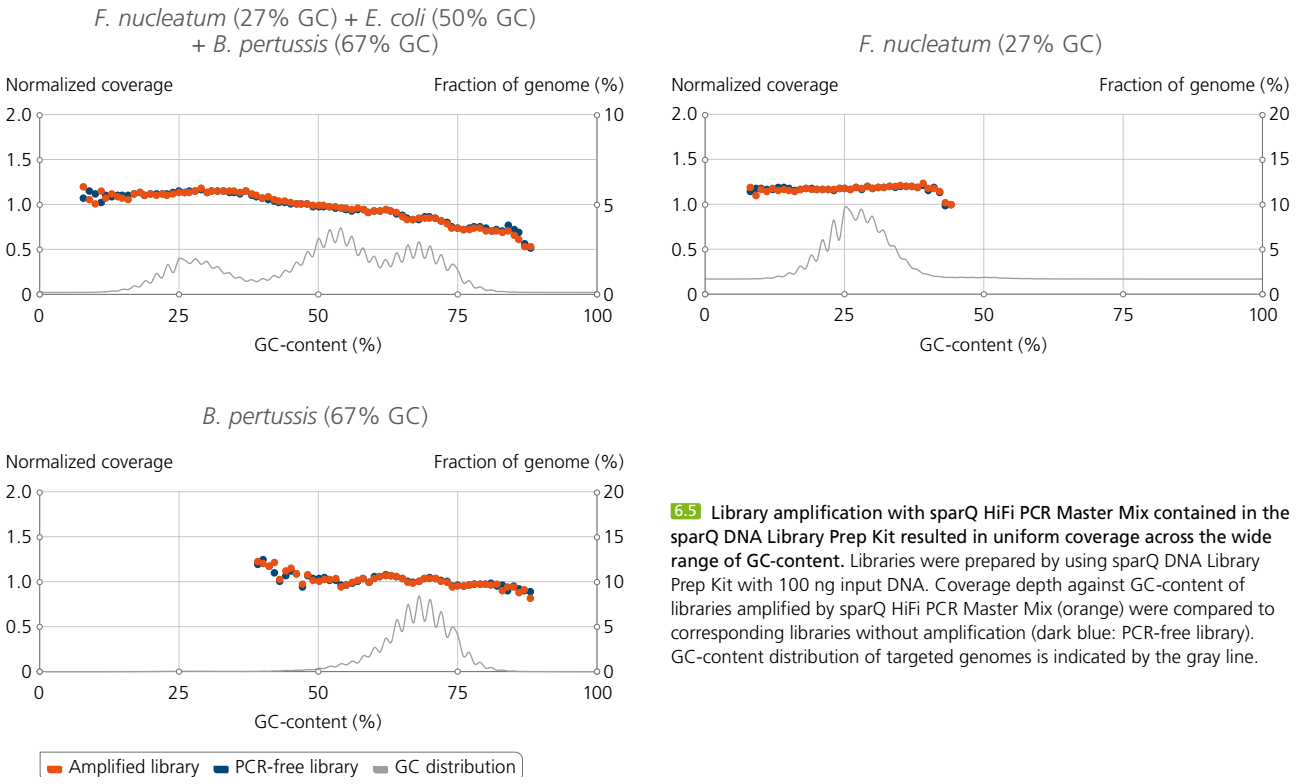
Even coverage across a broad range of GC-content

The sparQ DNA Library Prep Kit enables the construction of high quality libraries with uniform coverage across a wide range of GC-content. For applications requiring amplification, the HiFi PCR Master mix is formulated to increase library yields while reducing the number of cycles required to create a sequencing-ready library, thereby minimizing PCR-derived artifacts.



6.4 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).

Coverage of amplified library prepared with sparQ DNA Library Prep Kit closely resembles coverage of PCR-free workflows, both indicating good representation of GC- and AT-rich regions in the final library. Even coverage ensures greater sequencing depth or multiplexing capabilities.



6.5 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 the gray line.

Improved sequencing results

Whether using an amplified or a PCR-free workflow, sparQ DNA Library Prep Kit produces industry leading sequencing results as determined by the high number of reads mapping back to the reference genome and minimal duplication rates.

	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
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)
sparQ PureMag Beads - 5 ml	95196-005	5 ml
sparQ PureMag Beads - 60 ml	95196-060	60 ml
sparQ PureMag Beads - 450 ml	95196-450	450 ml
sparQ Universal Library Quant Kit - 100	95210-100	100 rxns
sparQ Universal Library Quant Kit - 500	95210-500	500 rxns

sparQ UDI Adapters (1–96)

Dual-indexed barcoded adapters for DNA and RNA libraries for Illumina

FEATURES AND BENEFITS:

- Flexible pooling: multiplex up to 96 samples per sequencing run
- Improved performance: prevents index hopping and enhances demultiplexing accuracy
- Optimized: for maximum ligation efficiency with sparQ library prep kits
- Multiple applications: including whole genome (with amplification or PCR-free), target enrichment, whole transcriptome sequencing and many more

DESCRIPTION:

sparQ UDI Adapters (1-96) are unique dual-indexed adapters designed to be used on Illumina sequencing platforms. It is compatible with both DNA and RNA libraries. The use of UDI's minimizes index hopping events while also enabling greater multiplexing on flow cells.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
sparQ UDI Adapters (1-96)	95211-096	1–96

sparQ DNA Frag & Library Prep Kit

Integrated enzymatic fragmentation and library prep with unrivaled speed and performance

FEATURES AND BENEFITS:

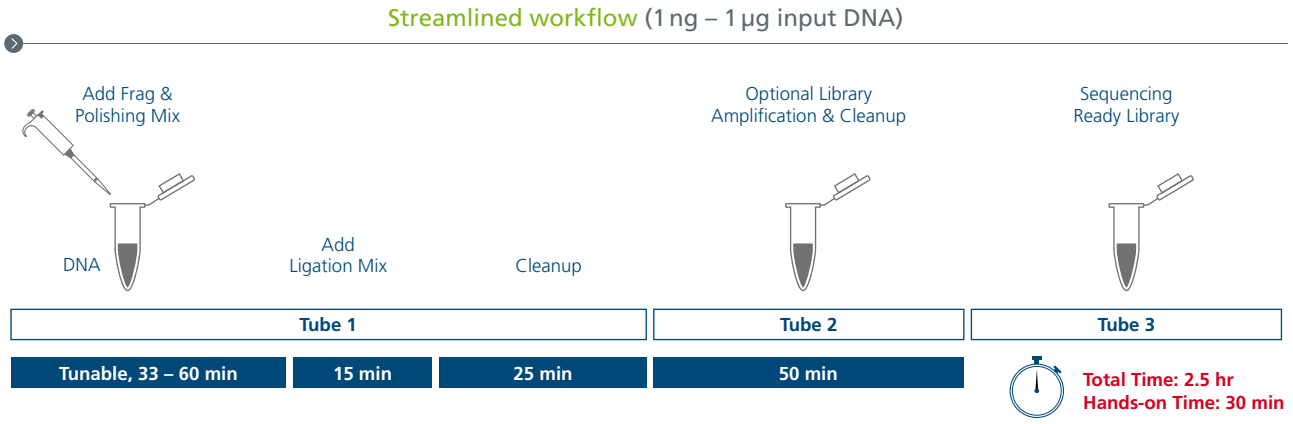
- High quality libraries in 2.5 hours from 1 ng – 1 µg of input DNA
- Tunable and reproducible fragmentation size range
- Simple, convenient 2-step workflow with minimal hands-on time
- Novel chemistry and high-fidelity amplification minimizing bias
- Superior sequence coverage uniformity and low duplication rate

DESCRIPTION:

The sparQ DNA Frag & Library Prep Kit is optimized for enzymatic fragmentation of DNA and streamlined construction of high quality libraries for sequencing on Illumina NGS platforms. The simple, convenient 2-step workflow can be completed in 2.5 hours with minimal hands-on time and accommodates DNA input amounts from 1 ng to 1 µg.

Quantabio's engineered DNA frag and polishing enzymes work in concert to generate fragment sizes that are tunable and reproducible based on reaction time. The DNA fragmentation and polishing reactions are combined in a single step to generate 5'-phosphorylated and 3'-dA-tailed fragments. This minimizes over fragmentation while greatly simplifies the library prep workflow. Subsequent high efficiency ligation of adapters is performed in the same tube without an intervening cleanup step. If library amplification is required, the HiFi PCR Master Mix and Primer Mix ensure even amplification with minimal bias.

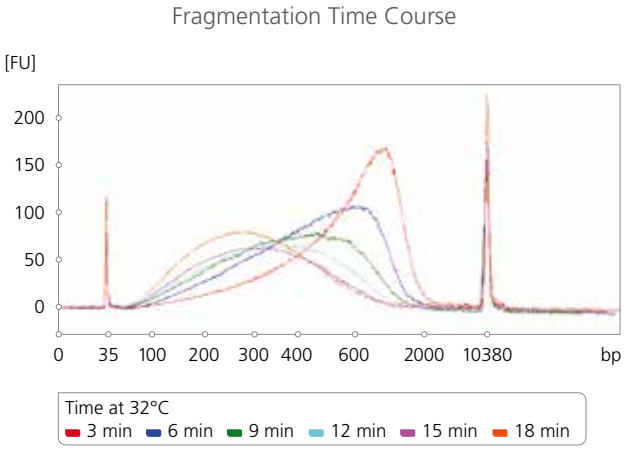
This kit is compatible with single-indexed, or dual-indexed Y-shaped adapters routinely used in library construction.



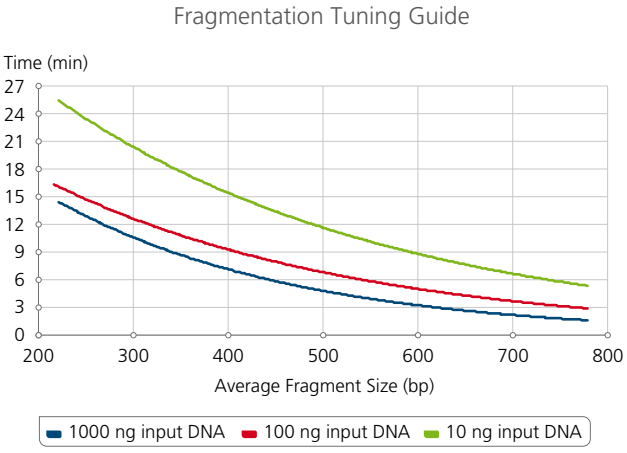
6.6 **The streamlined workflow utilizes a proprietary enzyme mix that combines fragmentation and DNA polishing in a single step to simplify library construction.** High efficiency adapter ligation and cleanup are performed in the same tube, followed by an optional amplification step using HiFi PCR Master Mix and Primer Mix.

Tunable & reproducible fragmentation

The sparQ DNA Frag & Library Prep kit is designed to produce consistent and reproducible fragments that are tunable to application-specific sizes. The fragmentation profile closely resembles Covaris mechanical shearing. Flexible input DNA amounts ranging from 1 ng – 1 µg can be accommodated. The single-tube enzyme mix fragments DNA and then automatically proceeds to the DNA polishing reaction, thus minimizing potential over fragmentation. Guidelines of incubation time and expected size based on input amount are provided below.



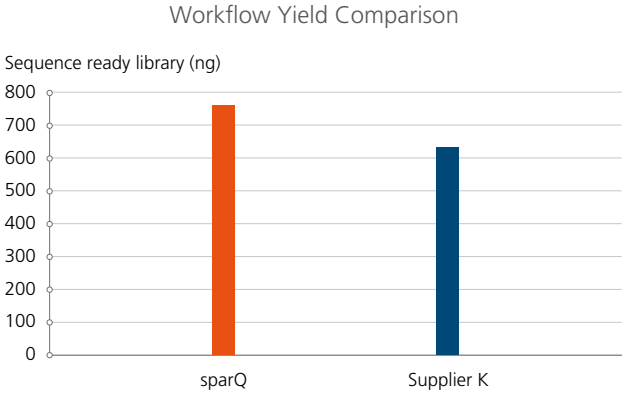
6.7 sparQ DNA Frag & Library Prep Kit is tunable to the desired fragment size. 100 ng Human gDNA was subjected to fragmentation with a series of incubation time points (3 – 18 min). After fragmentation, DNA samples were purified and then visualized using an Agilent High Sensitivity DNA Kit.



6.8 Guidelines for tuning fragmentation size. If input DNA falls between values displayed on the graph, an estimate can be used for optimizing fragmentation times.

Superior library prep efficiency and yields

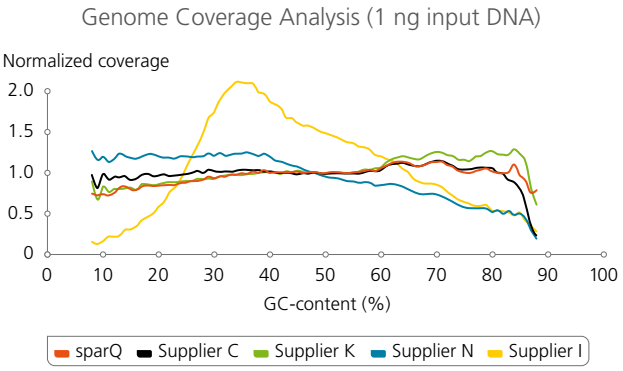
The novel and optimized chemistry used in the sparQ DNA Frag & Library Prep Kit coupled with proprietary enzyme mix lead to better sensitivity and higher library yields. PCR-free workflows are enabled for 100 ng of input DNA. For applications requiring amplification, the HiFi PCR Master Mix and Primer Mix allow researchers to achieve target concentration with very few cycles thereby reducing PCR-derived artifacts. Ultimately, precious samples can be saved for additional applications when necessary.

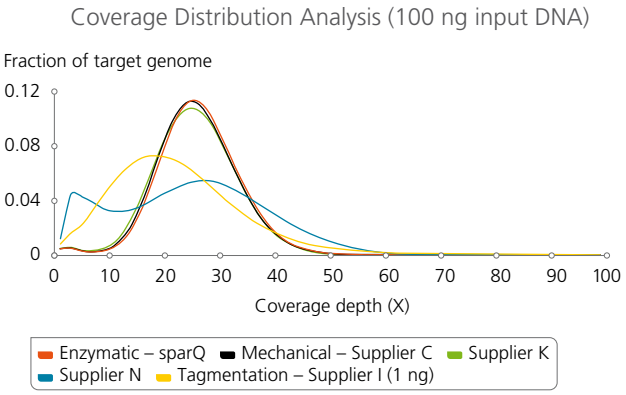


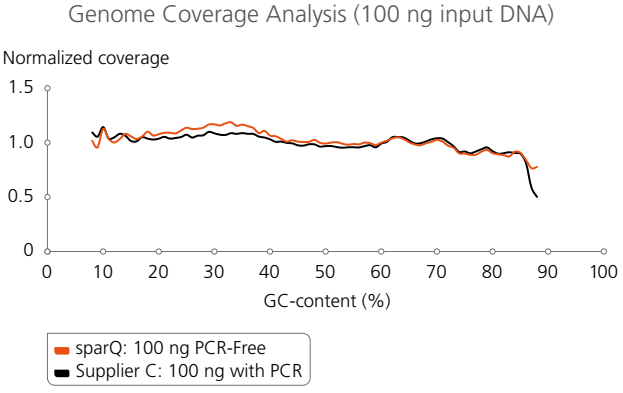
6.9 sparQ DNA Frag & Library Prep Kit shows significantly higher NGS library preparation efficiency. Libraries with 300 bp average DNA fragments from 100 ng of gDNA Coriell NA12878 were prepared using sparQ DNA Frag & Library Prep Kit and a commercial kit from Supplier K. Manufacturers' manuals were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

Uniform coverage across a wide GC-spectrum

The sparQ chemistry enables high quality library construction with even coverage across a board GC-spectrum including challenging GC- and AT-rich regions. Reproducible and uniform genome coverage is achieved independent of input DNA amounts, comparable to coverage obtained by mechanical shearing workflows. The sparQ DNA Frag & Library Prep Kit ensures similar total coverage depth for the majority of genomic targets, thus reducing the need for additional sequencing, resulting in less sequencing per sample and lower total sequencing costs.







6.10 **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 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 mechanical shearing method.

High quality sequencing metrics with low duplication rates

Excellent sequencing metrics – high mapping percentage and low duplication artifacts – are achieved with the sparQ DNA Frag & Library Prep Kit, ensuring the greatest return on sequencing investments.

		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
sparQ DNA Library Prep Kit - 24	95191-024	24 rxns
sparQ DNA Library Prep Kit - 96	95191-096	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)
sparQ PureMag Beads - 5 ml	95196-005	5 ml
sparQ PureMag Beads - 60 ml	95196-060	60 ml
sparQ PureMag Beads - 450 ml	95196-450	450 ml
sparQ Universal Library Quant Kit - 100	95210-100	100 rxns
sparQ Universal Library Quant Kit - 500	95210-500	500 rxns

sparQ PureMag Beads

Fast, reliable DNA purification & size selection for NGS workflows

FEATURES AND BENEFITS:

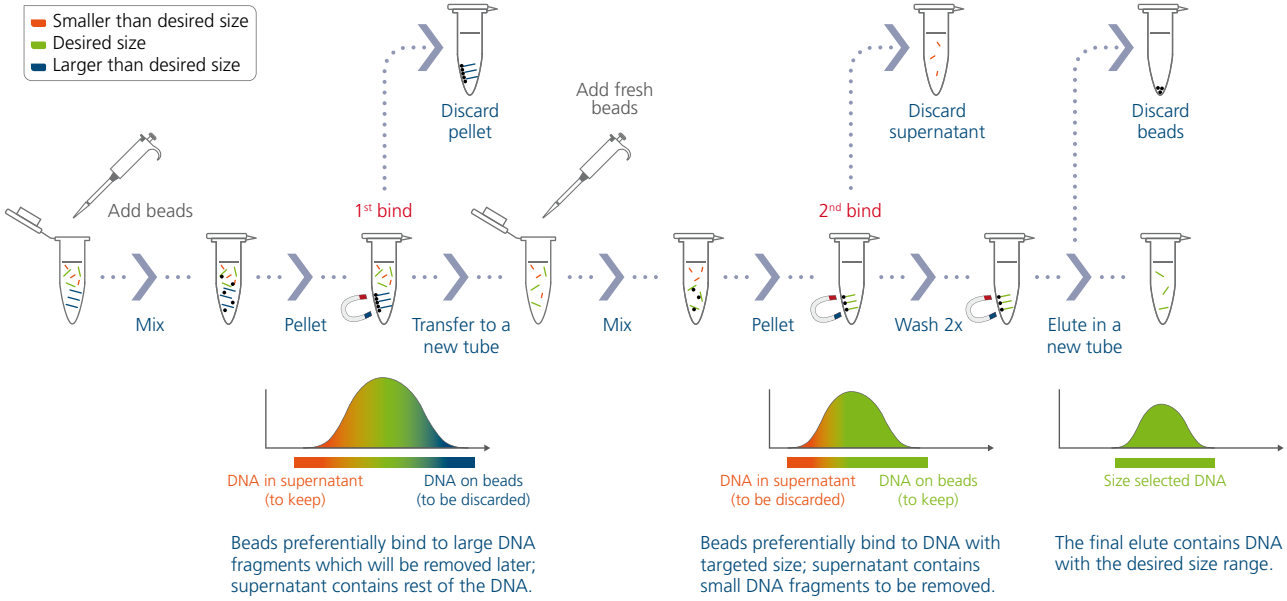
- High recovery of DNA fragments greater than 100 bp
- Efficient removal of unwanted components from adapter ligation and PCR reactions
- Consistent single or double-sided size selection
- Seamless integration into existing NGS workflows with little or no protocol change

DESCRIPTION:

sparQ PureMag Beads is a fast and reliable nucleic acid purification system for reaction cleanup and size selection in Next Generation Sequencing (NGS) workflows. Based on the reversible nucleic acid-binding properties of magnetic beads, this product can be used to quickly remove primers, primer-dimers, unincorporated nucleotides, salts, adapters and adapter-dimers from NGS library prep reactions to improve downstream sequencing performance.

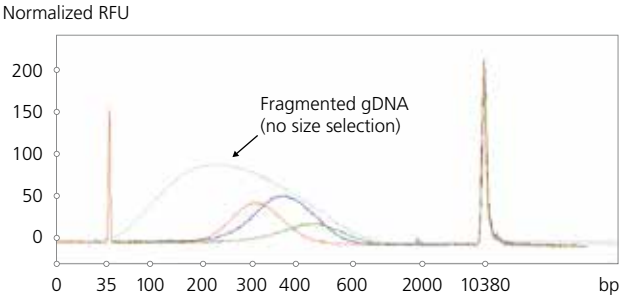
sparQ PureMag Beads allows excellent recovery of fragments greater than 100 bp without centrifugation or filtration. Consistent and reliable size selection can be achieved by simply adjusting the beads to sample ratio. This product is designed for both manual and automated processing, allowing seamless integration into existing workflows.

sparQ PureMag Beads workflow



6.11 **Double-sided size selection is used to remove smaller and larger fragments from either side of the desired region.** The fragment size can be easily adjusted to suit the application by manipulating the sparQ PureMag Beads to DNA volumetric ratio.

Bioanalyzer trace of fragmented human genomic DNA pre- and post double-sided size selection

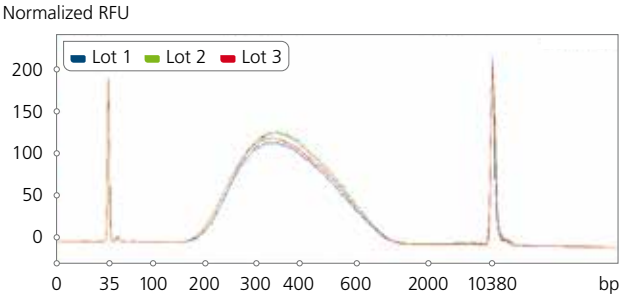


sparQ PureMag Beads DNA ratio	Targeted size range (bp)	Peak average (bp)
0.7x, 0.9x	200–400	301
0.6x, 0.8x	250–500	377
0.5x, 0.7x	300–700	464

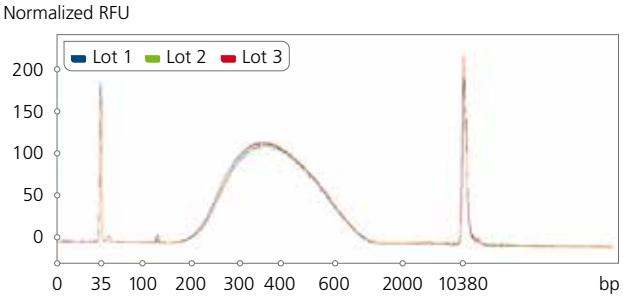
6.12 Electropherogram of fragmented human genomic DNA pre- and post double-sided size selection. Different sparQ PureMag Beads to DNA ratios were used to achieve various targeted size range.

Highly reproducible purification across a range of inputs

DNA library profile (100 ng input)

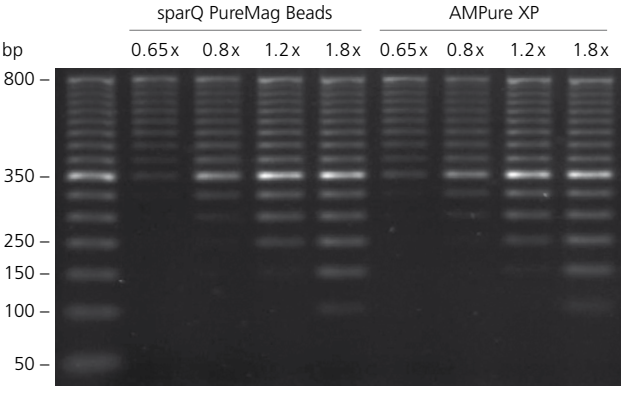


DNA library profile (1 ng input)



6.13 Highly reproducible DNA library profiles were achieved using different lots of sparQ PureMag Beads and a broad range of input amount. Libraries were prepared with sparQ DNA Library Prep Kit from 100 ng and 1 ng of fragmented microbial genomic DNA. sparQ PureMag Beads were used post adapter ligation and PCR amplification to effectively remove adapter-dimers and primer-dimers.

Efficient recovery of DNA equivalent to AMPure XP



6.14 sparQ PureMag Beads show equivalent performance to AMPure XP for DNA purification. 50 bp DNA ladder was purified with sparQ PureMag Beads and AMPure XP at different beads to DNA ratios and analyzed on 2% agarose gel.

ORDER INFO

Product Name	Quantabio Catalog Number	Size
sparQ PureMag Beads - 5 ml	95196-005	5 ml
sparQ PureMag Beads - 60 ml	95196-060	60 ml
sparQ PureMag Beads - 450 ml	95196-450	450 ml

sparQ HiFi PCR Master Mix

High-fidelity library amplification while maintaining even coverage

FEATURES AND BENEFITS:

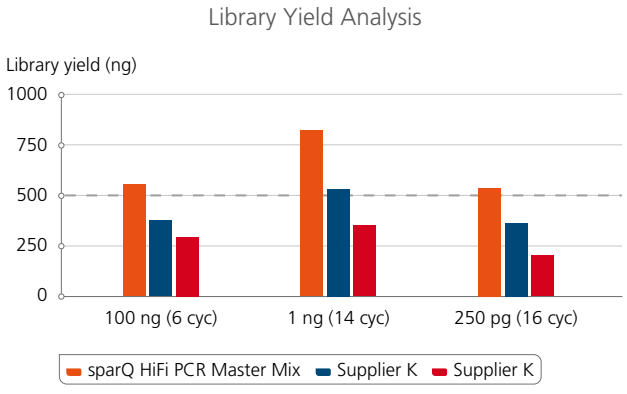
- HiFi DNA polymerase engineered to minimize amplification bias
- Increased amplification efficiency resulting in higher yields
- Uniform coverage across challenging AT- and GC-rich regions
- Robust amplification from input DNA as low as 250 pg

DESCRIPTION:

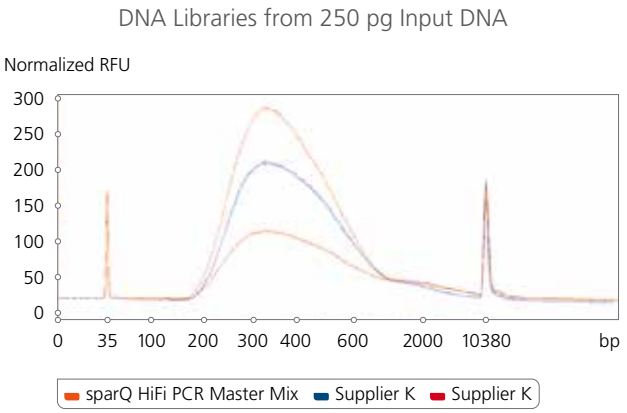
The sparQ HiFi PCR Master Mix is a high-fidelity, high-efficiency PCR master mix for NGS workflows requiring DNA library amplification prior to sequencing. The included primer mix allows amplification of DNA libraries flanked by adapters containing the P5 and P7 sequences required for Illumina sequencing platforms. The hot-start, proofreading DNA polymerase used in the sparQ HiFi PCR Mater Mix is specifically engineered to improve library amplification efficiency while reducing PCR-derived artifacts, resulting in higher library yields and better coverage uniformity. This kit supports low DNA input from 250 pg and efficient amplification of AT- and GC-rich regions with minimal bias.

Higher library amplification efficiency

Specially designed for sensitive, high efficiency library amplification from a broad range of DNA input, the sparQ HiFi PCR Master Mix minimizes the number of amplification cycles needed to achieve the threshold required for sequencing. The result is >45% higher library yields while reducing PCR-derived artifacts.



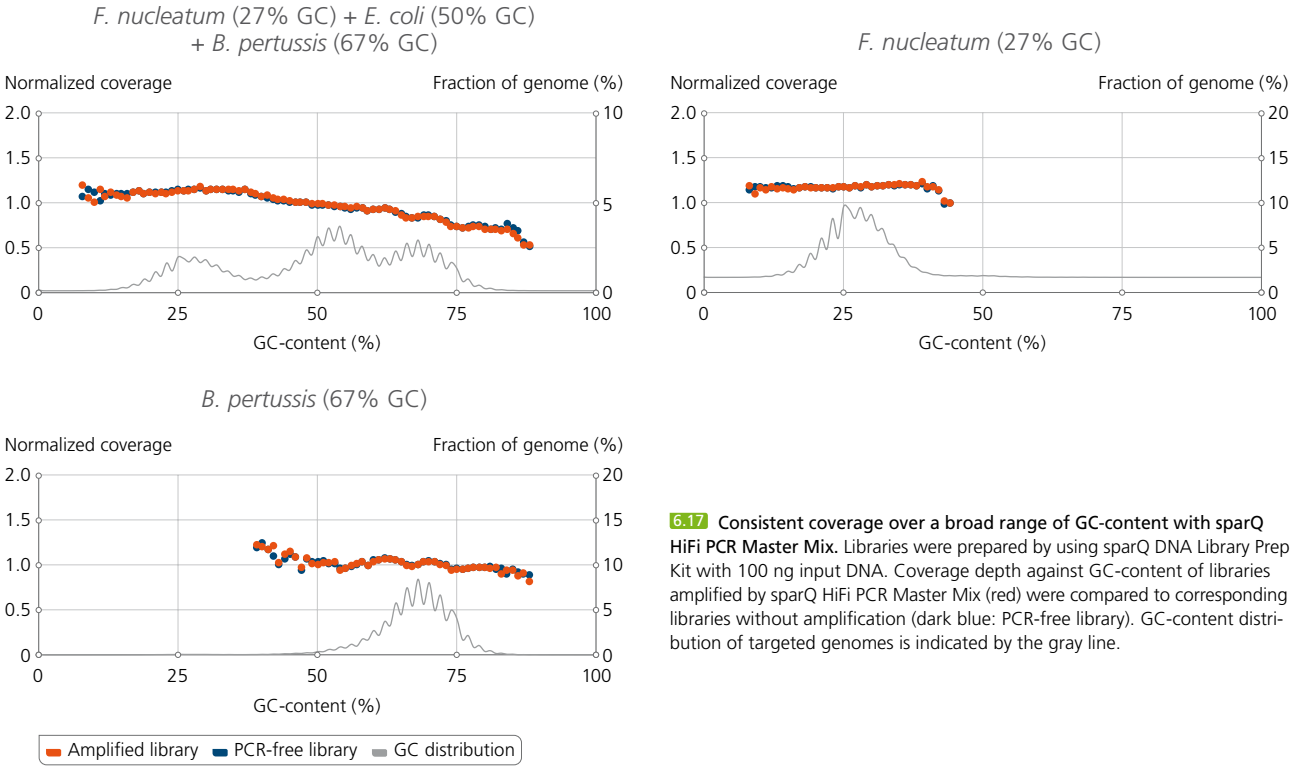
6.15 Library amplification with sparQ HiFi PCR Master Mix resulted in higher yields. Libraries were prepared from Covaris-sheared human genomic DNA with sparQ DNA library prep kit prior to library amplification. Pre-amplified libraries were then amplified using sparQ HiFi PCR Master Mix (orange) or equivalent kit from Supplier K (blue) and Supplier N (red) with identical PCR 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 method and qPCR-based quantification method (data not shown).



6.16 sparQ HiFi PCR Master Mix demonstrates high efficiency library amplification from low input. 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 the Agilent BioAnalyzer. Libraries were amplified using sparQ HiFi PCR Master Mix (orange) or equivalent kit from Supplier K (blue) and Supplier N (red) with identical amplification cycle numbers (16 cycles for 250 pg input DNA).

Superior coverage uniformity

Libraries amplified by sparQ HiFi PCR Master Mix provide uniform coverage across a broad range of GC-content, similar to corresponding libraries without PCR. Even coverage ensures greater sequencing depth or multiplexing capabilities.



6.17 **Consistent coverage over a broad range of GC-content with sparQ HiFi PCR Master Mix.** 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 the 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)
Related Products		
sparQ DNA Frag & Library Prep Kit - 24 R	95194-024	24 rxns
sparQ DNA Frag & Library Prep Kit - 96 R	95194-096	96 rxns
sparQ DNA Library Prep Kit - 24 R	95191-024	24 rxns
sparQ DNA Library Prep Kit - 96 R	95191-096	96 rxns
sparQ PureMag Beads - 5 ml	95196-005	5 ml
sparQ PureMag Beads - 60 ml	95196-060	60 ml
sparQ PureMag Beads - 450 ml	95196-450	450 ml
sparQ Universal Library Quant Kit - 100	95210-100	100 rxns
sparQ Universal Library Quant Kit - 500	95210-500	500 rxns

sparQ Universal Library Quant Kit

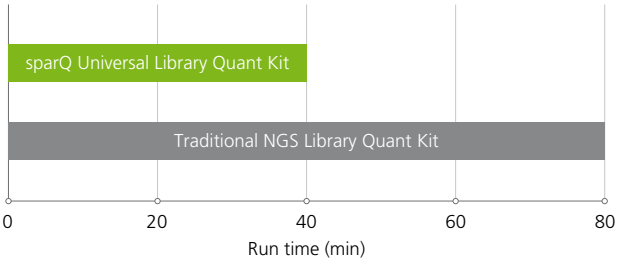
Fastest qPCR-based library quantification in 40 minutes

FEATURES & BENEFITS:

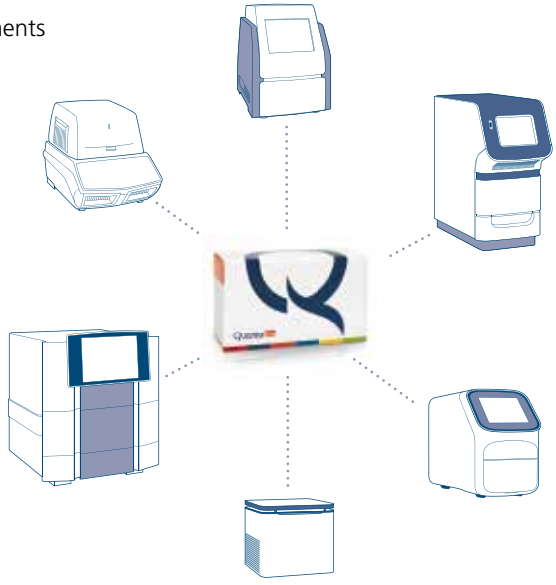
- Faster time to results – 50% shorter run time than traditional cycling protocols
- Accurate, reliable quantification of NGS libraries of various sizes and GC-content
- High amplification efficiency across a wide linear dynamic range
- Stabilized, ready-to-use sparQ Universal Fast Mastermix to reduce pipetting steps
- Superior run-to-run uniformity ensuring highly precise measurements

DESCRIPTION:

sparQ Universal Library Quant Kit provides rapid and accurate quantification of libraries prepared for sequencing on Illumina NGS platforms. Accurate quantification of the number of amplifiable library molecules prior to loading onto a flow cell is a critical step in the NGS workflow and it ensures optimal cluster generation and cost-effective use of sequencing capacity. The sparQ Universal Library Quant Kit uses real-time quantitative PCR (qPCR) to specifically quantify the number of library molecules that possess the appropriate adapter tag at each end.



6.18 **Comparison of average qPCR run time for library quantification.** sparQ Universal Library Quant Kit uses a fast cycling protocol, allowing results to be achieved in 40 minutes versus 80 minutes with traditional NGS Library Quant Kit.



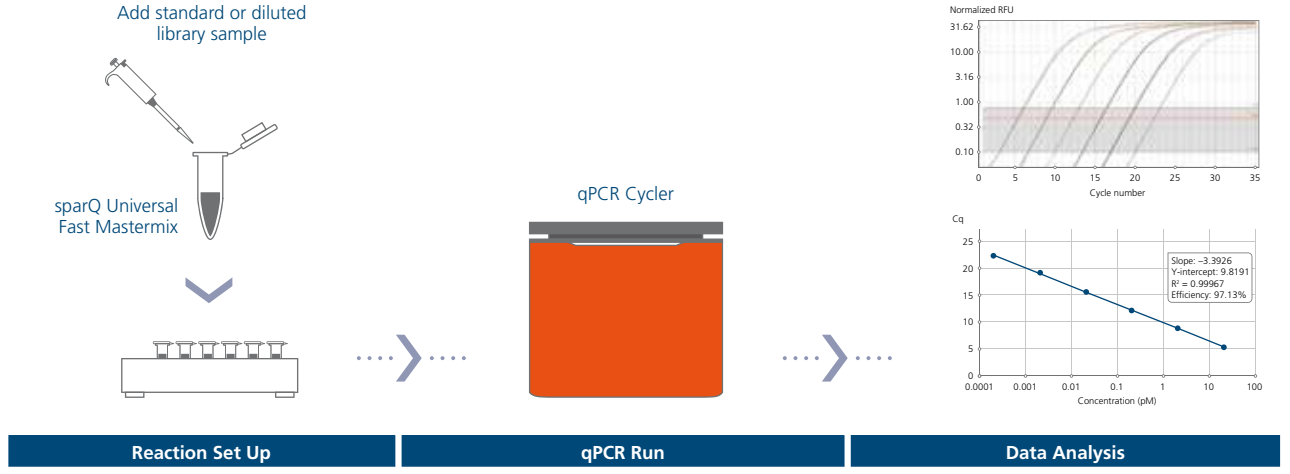
Accurate library quantification in 40 minutes

This kit is optimized for use on any qPCR instrument whether or not the ROX reference dye is required. The sparQ Universal Library Quant Kit employs a proprietary, fast taq-based mastermix that enables fast cycling, reducing qPCR run time by 50% compared to traditional cycling protocols.

Complete library quantification solution with unmatched convenience

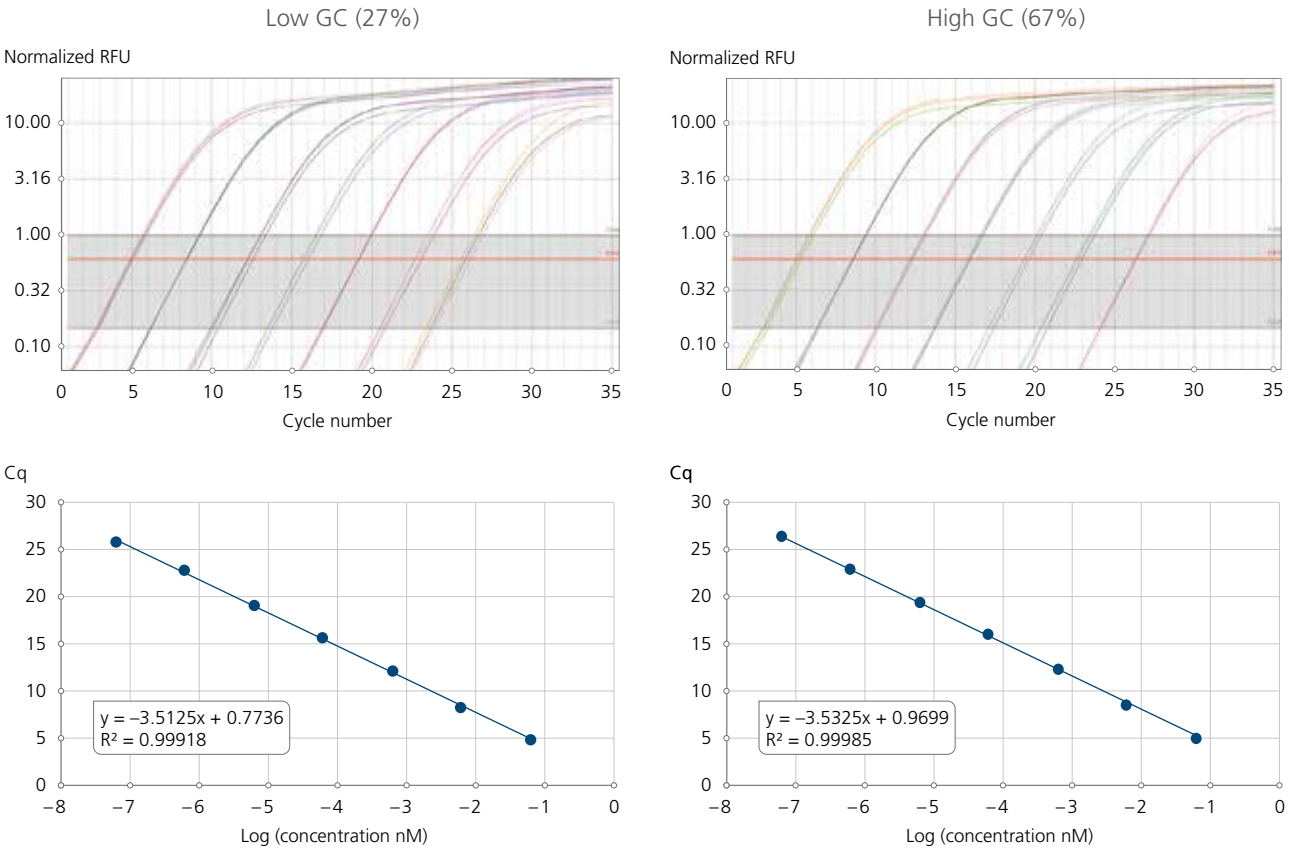
sparQ Universal Library Quant Kit contains six stabilized, pre-diluted DNA standards, ready-to-use 1.25x mastermix pre-mixed with primer sets containing Illumina P5 and P7 sequences, and an optimized buffer for diluting NGS library samples. The mastermix is ready-to-use for qPCR instruments with no and low ROX requirements. A tube of ROX is included in the kit for qPCR instruments requiring higher concentrations of the reference dye. This unique formulation minimizes pipetting steps and ensures precise qPCR results.

sparQ Universal Library Quant Kit Workflow



6.19 Illustration of sparQ Universal Library Quant Kit workflow. Reactions are prepared by simply adding standard or diluted library sample. Optimized protocols with fast cycling condition are provided for both 10 µl or 20 µl reaction volumes.

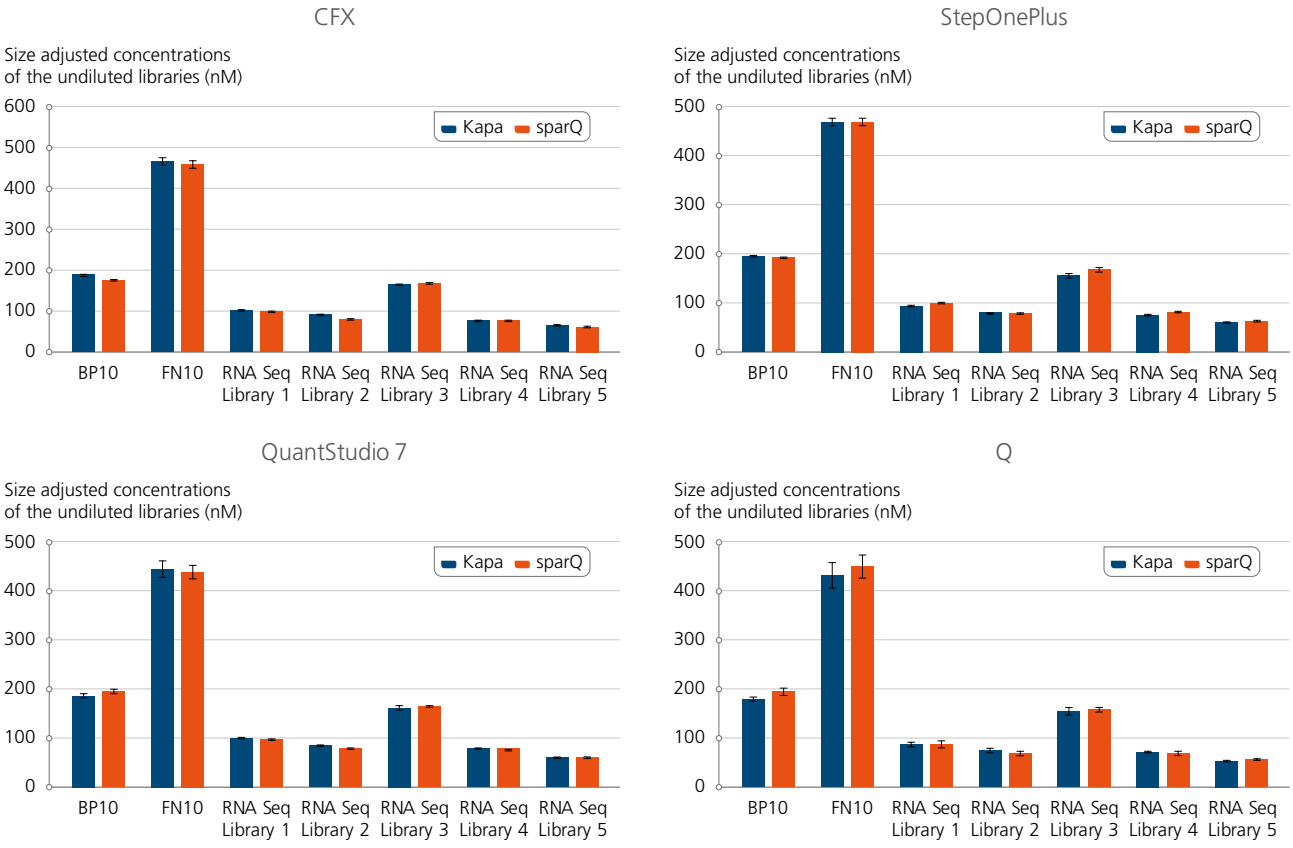
High amplification efficiency across a wide linear dynamic range



6.20 **sparQ Universal Library Quant Kit provides high amplification efficiency across a wide linear dynamic range.** A 10-fold dilution series was prepared from libraries of low (27%) and high (67%) GC-content and amplified under fast conditions on the Quantabio Q qPCR cycler using the sparQ Universal Fast Mastermix. The slopes of the Cq vs Log (concentration) plots and the individual sample reactions measured by the LinRegPCR algorithm indicated superb amplification efficiencies.

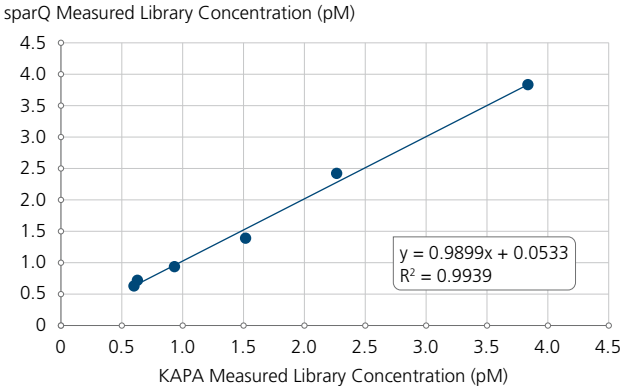
Equivalent performance across cyclers: sparQ vs Kapa Library Quantification Kit

sparQ Universal Quant kits provides concordant library quantification calls when compared with Kapa Library Quantification Kit across a range of different qPCR cyclers as well as types of nucleic acids. sparQ also provides library quantification calls in 40 minutes vs 80 minutes with Kapa.



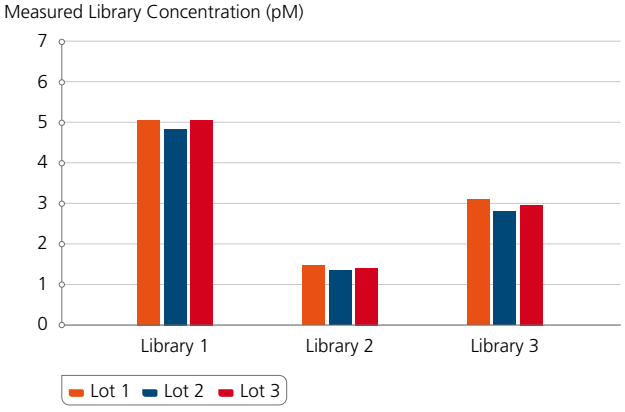
6.21 **Seven Libraries (2 DNA, 5 RNA) were prepared and each quantified using both sparQ Universal Library Quant Kit and Kapa™ Library Quantaification Kit according to each manufacturer's cycling protocol.** Both kits produced library quantification results that were concordant to one another across a range of real time qPCR cyclers (BioRad® CFX, Applied Biosystems StepOne Plus™, Applied Biosystems QuantStudio™ 7 and Quantabio Q).

Equivalent performance with 50% faster run time



6.22 Results from sparQ and Roche KAPA Library Quantification Kits were highly correlated. Concentrations of six different diluted libraries were determined using either the sparQ Universal Library Quant Kit on Q or the Roche KAPA Library Quantification Kit on Bio-Rad CFX following the manufacturer's recommended protocol. Run times, including melt curves, were 40 minutes for sparQ and 80 minutes for KAPA.

Lot-to-lot consistency of sparQ DNA Standards



6.23 sparQ Universal Library Quant Kits are manufactured with high lot to-lot consistency. Concentrations of diluted libraries with low GC (library 1), high GC (library 2), or balanced GC-content (library 3) were determined using 3 different lots of sparQ DNA Standards. Each library sample was tested in quadruplicate reactions with each lot of sparQ DNA Standards. Standard deviations of average quantification values were all <0.13 pM.

ORDER INFO

Product Name	Quantabio Catalog Number	Size*
sparQ Universal Library Quant Kit - 100	95210-100	100 rxns
sparQ Universal Library Quant Kit - 500	95210-500	500 rxns

*** Based on 20 µl reaction volume.**

7.0 qPCR Instrument

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/cycler)
- **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 as 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.

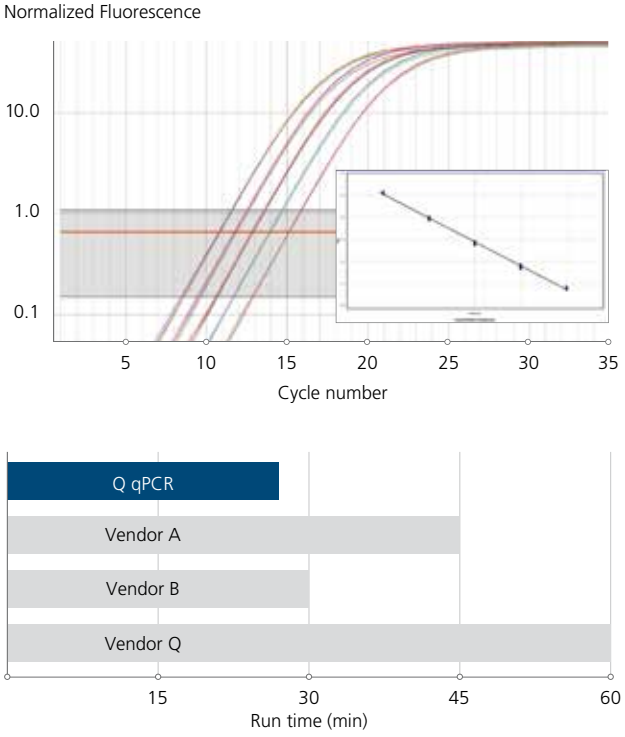
7.1 Q qPCR cycler.

* 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



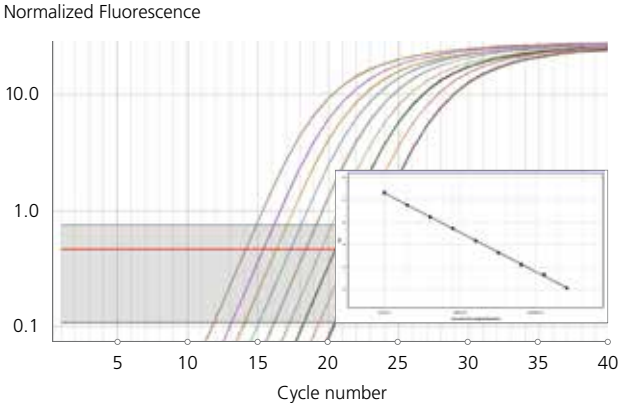
7.2 5 point, 2x dilution series of Hepatitis B virus (HBV) cDNA template. Starting amount of 3E+06 copies (n = 4 each); Efficiency = 90% (standard curve method); R^2 = 0.99; Time to complete run (including melt) = 26 min

*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



7.3 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 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.

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

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