SensiFAST™ qPCR Guide

Superior Fast Gene Expression Analysis

meridian BIOSCIENCE®
LIFE DISCOVERED. LIFE DIAGNOSED.

bioline.com/sensifast

Quantitative PCR

Quantitative PCR (qPCR) is one of the most powerful and sensitive gene analysis techniques available. The main advantage of qPCR over traditional end-point PCR is that it allows you to determine the starting template copy number of your DNA or cDNA with accuracy and high sensitivity over a wide dynamic range. Most modern PCR applications are qPCR based and can be used in diagnostic, agricultural, biotechnology and pharmaceutical research.

Gene Expression Using qPCR - Technical Considerations

Although qPCR is considered the gold standard for accurate measurement of gene expression, the true accuracy and subsequent usability of qPCR data is greatly dependent on experimental design, overall workflow and analysis techniques.

There are a number of critical issues across the entire workflow from sample selection and processing, use of appropriate controls and calculation methods, as well as overall experimental design and reporting strategies. Meridian recommends complying with the MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) Guidelines* during qPCR experimental design.

Table of Contents | Page

*The MIQE Guidelines: (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) Guidelines. S. Bustin et al., Clinical Chemistry 55:4, 611-622 (2009)

SensiFAST Selection Table

Manufacturer	Model	Lo-ROX	Hi-ROX	No-ROX	HRM Compatible
Agilent (Stratagene)	AriaMX	✓			✓
	MX3000P™, MX3005P™, MX4000P™	✓			✓
Analytika Jena	qTower, qTower 2.x			✓	
Applied Biosystems™	7000		✓		
	7300		✓		
	7500	✓			
	7500 FAST	✓			✓
	7700		✓		
	7900		✓		
	7900 HT		✓		
	7900HT FAST	✓			✓
	Quantstudio™ 3,5,6,7, 12k flex	✓			✓
	StepOne™		✓		✓
	StepOne™ Plus		✓		✓
	Viia7™	✓			✓
Bio-Rad®	CFX96™			✓	✓
	CFX384™			✓	✓
	Chromo4™			✓	
	iCycler®	✓			
	iQ™5			✓	
	MiniOpticon™			✓	
	MyiQ™			✓	
	Opticon™			✓	
	Opticon™2			✓	
BJS	Xxpress®			✓	
BMS	MIC			✓	✓
Cepheid®	SmartCycler®			✓	
Eppendorf	Mastercycler® ep realplex			✓	✓
	Mastercycler® ep realplex 2S			✓	✓
Fluidigm	BioMark™	✓			
IT-IS Life Science	MyGo Pro			✓	✓
PCRmax	Eco™			✓	✓
Qiagen	Rotor-Gene™ 3000			✓	
	Rotor-Gene™ 6000			✓	✓
	Rotor-Gene™ Q			✓	✓
Roche	Lightcycler®96			✓	✓
	Lightcycler®480			✓	✓
	Lightcycler®Nano			✓	✓
Takara	Thermal Cycler Dice®			✓	
Techne	PrimeQ			✓	
	Quantica®			✓	
Thermo	Piko Real™			✓	

One-Step qPCR Kits

SensiFAST™ One-Step RT-qPCR Kits are available in a variety of configurations to suit all of your different applications and techniques.

SensiFAST One-Step Kits are a complete range of highly-optimized ready-to-use kits, designed for first-strand cDNA synthesis and subsequent qPCR amplification of a specific target RNA, from either total RNA or mRNA, in a single tube. This reduces sample handling, which helps to decrease the chances of pipetting errors and cross contamination, as well as reduced bench time, meaning faster time to results. In contrast to two-step protocols, gene specific primers are required and all of the cDNA is consumed in the qPCR step.

SensiFAST benefits from the latest developments in real-time reverse transcription qPCR (RT-qPCR) to realize the fastest cycling times and greatest sensitivity, without compromising accuracy reproducibility or performance. SensiFAST One-Step Kits can be used on all qPCR instruments and are ideal for the new generation of fast PCR cyclers (see selection table).

SensiFAST One-Step Kits provide the perfect solution when processing multiple samples and amplifying only a few genes of interest, making SensiFAST One-Step Kits ideal for assays such as virus detection and quantification and high-throughput gene expression screening, without compromising on sensitivity and reproducibility (see Table 1).

- **Sensitive:** optimized buffer formulation delivers reliable quantification from even very low-copy number RNA targets
- **Reproducible:** consistent results between technical replicates for increased accuracy
- **Specific:** antibody-mediated hot-start DNA polymerase minimizes non-specific amplification for improved assay sensitivity and reliability
- **Robust:** reliable detection of RNA targets from a broad range of sample types
- **Fast:** optimized proprietary mix of enzymes and RT buffer chemistry, delivers reproducible, accurate assay results in as little as 40 minutes

Table 1 Applications for SensiFAST One-Step kits

Gene expression analysis	Microarray validation	Viral quantitation
Pathogen detection	Biomarker discovery and validation	Gene knockdown validation
Genotyping	Cellular mRNA and miRNA	ChIP
Gene dosage determination	Cancer risk assessment	Microbial quantification
Detection of extremely low copy targets	Quantification	Drug therapy efficacy

SensiFAST™ SYBR® One-Step Kit

SensiFAST SYBR® One-Step Kit has been formulated for fast, efficient, unbiased cDNA synthesis and subsequent highly-sensitive (Fig. 1), reproducible RT-qPCR in a single tube (Fig. 2). An antibody-mediated hot-start DNA polymerase promotes rapid activation and supports highly-specific amplification, which in turn improves assay sensitivity and dynamic range. A combination of the latest advances in buffer chemistry and PCR enhancers confer superior assay performance under fast thermal cycling conditions. The inclusion of separate RiboSafe Inhibitor ensures accuracy by protecting RNA targets from RNase degradation.

SensiFAST SYBR® One-Step Kit consists of a 2x SensiFAST SYBR® One-Step mix, a separate reverse transcriptase and RiboSafe RNase Inhibitor and has been validated on all commonly used qPCR instruments.

> *"SensiFAST Probe One-Step Kit has provided our lab with a fast, reliable and economic solution for our viral testing qPCR assays. Our lab routinely uses a quadruplex reaction and SensiFAST is the only mix where we achieve the same results as with a singleplex reaction."*
>
> *Center for Vectorbourne Diseases, University of California Davis, USA*

SYBR	Size	Cat. #
SensiFAST SYBR No-ROX One-Step Kit	100 Reactions	BIO-72001
	500 Reactions	BIO-72005
SensiFAST SYBR Hi-ROX One-Step Kit	100 Reactions	BIO-73001
	500 Reactions	BIO-73005
SensiFAST SYBR Lo-ROX One-Step Kit	100 Reactions	BIO-74001
	500 Reactions	BIO-74005

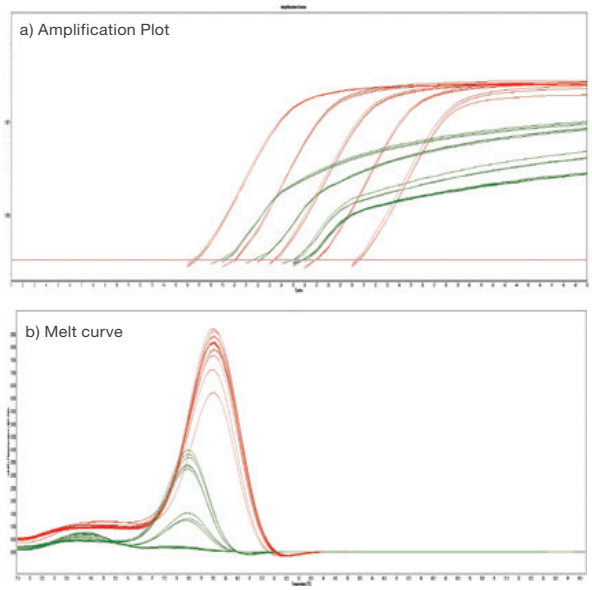


Fig. 1 Sensitivity under fast cycling conditions
A 10-fold serial dilution of human RNA (in triplicate) was amplified over 5 orders of magnitude, according to the manufacturers' standard protocol. The results illustrate that SensiFAST SYBR One-Step (red) could be diluted further than supplier T (green) and so is more sensitive.

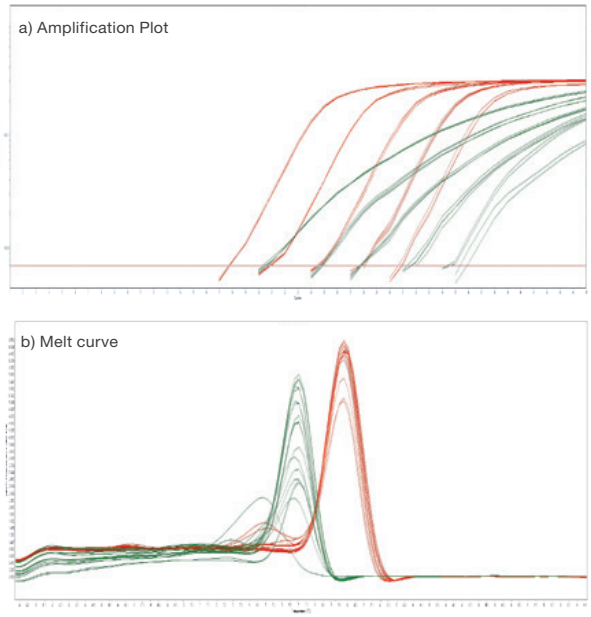


Fig. 2 Reproducibility under fast cycling conditions
A 10-fold serial dilution of human RNA (in triplicate) was amplified over 5 orders of magnitude, according to the manufacturers' standard protocol. The earlier Ct values illustrate that SensiFAST SYBR One-Step Kit (red) with tight replicates illustrating greater reproducibility than supplier Q (green).

SensiFAST™ Probe One-Step Kit

SensiFAST Probe One-Step Kit has been developed for fast RT-qPCR and designed for superior sensitivity (Fig. 1) and specificity (Fig. 2) with probe-detection technology, including TaqMan®, Scorpions® and molecular beacon probes. SensiFAST Probe One-Step Kit has been formulated for highly reproducible first-strand cDNA synthesis and subsequent RT-qPCR in a single tube. An antibody-mediated hot-start DNA polymerase promotes rapid activation and supports highly-specific amplification, which in turn improves assay sensitivity and dynamic range. A combination of the latest advances in buffer chemistry and PCR enhancers confer superior assay performance under fast thermal cycling conditions. This also gives SensiFAST Probe One-Step Kit unmatched efficiency in multiplexing (Fig. 3).

SensiFAST Probe One-Step Kit consists of a 2x SensiFAST Probe Mix, plus separate reverse transcriptase and RiboSafe RNase Inhibitor and has been validated on all commonly used qPCR instruments.

Probe	Size	Cat. #
SensiFAST Probe No-ROX One-Step Kit	100 Reactions	BIO-76001
	500 Reactions	BIO-76005
SensiFAST Probe Hi-ROX One-Step Kit	100 Reactions	BIO-77001
	500 Reactions	BIO-77005
SensiFAST Probe Lo-ROX One-Step Kit	100 Reactions	BIO-78001
	500 Reactions	BIO-78005

SensiFAST Probe One-Step Kit was used with human parainfluenzavirus type 4 to show sensitivity of down to 10 copies per reaction across an 8 log_{10} dynamic range.

University of Queensland, Australia

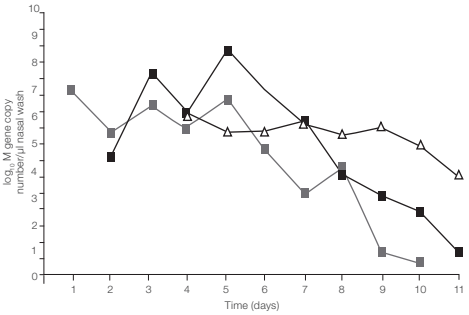


Fig. 1 Copy number of viral RNA in nasal washes
The WHO Collaborating Centre for Reference and Research on Influenza in Australia used RT-qPCR analysis of a mixed population of influenza viruses in ferret nasal washes to measure the viral replication and transmission kinetics of each virus population (*Butler et al 2014). The copy number of viral RNA in each nasal wash was determined over 11 days using SensiFAST Probe One-Step Kit. The results illustrate sensitivity of RT-qPCR, helping to show the fitness advantage conferred by mutations in drug-resistant influenza viruses.

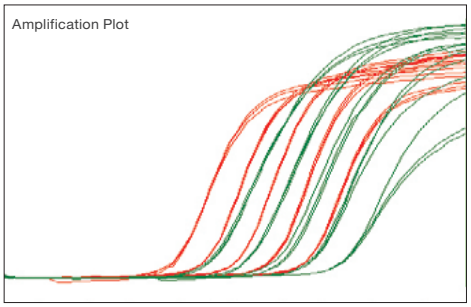


Fig. 2 Fast cycling conditions
Mouse ß-actin amplified in triplicate using gene specific primers and TaqMan probe according to each manufacturer's protocol, from 10-fold serial dilution of RNA with SensiFAST Probe One-Step and supplier A mix. The results illustrate that SensiFAST Probe One-Step Kit is faster than supplier A mix by four Ct (more than 10-fold).

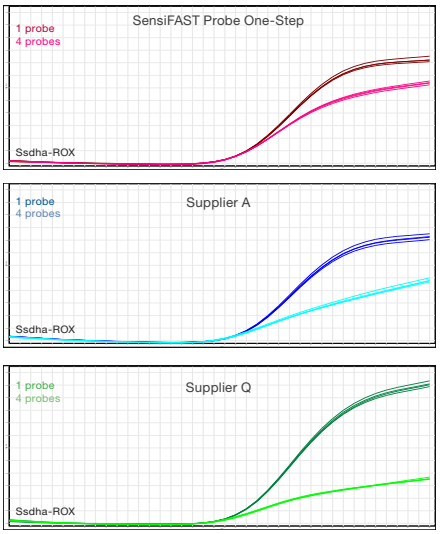


Fig. 3 Multiplexing performance comparison
A 100-fold dilution of human cDNA was used with four probes, either in singleplex reactions (darker line) or in quadruplex reaction (lighter line), using a conventional TaqMan prime/probe set. The results illustrate that the SensiFAST Probe One-Step Kit is very efficient in delivering the same Ct in singleplex and multiplex assays.

SensiFAST™ Probe Direct SuperMix

SensiFAST™ Probe Direct SuperMix is a highly inhibitor-resistant qPCR master mix that provides quick and easy extraction and amplification of DNA from a variety of tissue types. The supermix maximizes sensitivity while simultaneously minimizing the effect of blood, tissue and plant PCR inhibitors, to deliver greater experiment success rates. It has been designed for highly reproducible, accurate assay results in the presence of inhibitors, making it ideal for direct amplification from the most challenging samples.

The SensiFAST™ Probe Direct SuperMix is a combination of the latest advances in buffer chemistry and PCR enhancers and stabilizers, together with an antibody-mediated hot-start polymerase, dNTPs and $MgCl_2$.

Product	Size	Cat. #
SensiFAST Probe Direct SuperMix	500 Reactions	BIO-86105
	2000 Reactions	BIO-86120

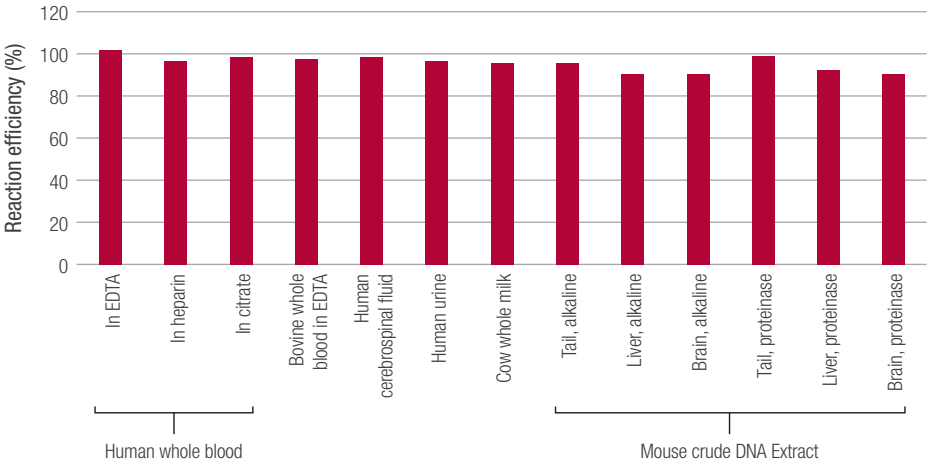


Fig. 1 Efficient amplification from different sample types
20% (final volume in reaction) samples of human whole blood containing anticoagulants (EDTA, Heparin and Citrate) and bovine whole blood (EDTA), human cerebrospinal fluid, human urine and cow whole milk were analyses using the SensiFAST Probe Direct SuperMix along with 2% alkaline or proteinase K mouse tail, liver and brain crude DNA extracts. The results illustrate that the reaction efficiency of the SensiFAST Probe Direct SuperMix remained within tolerances (90-110%) in the presence of a wide range of common PCR inhibitors.

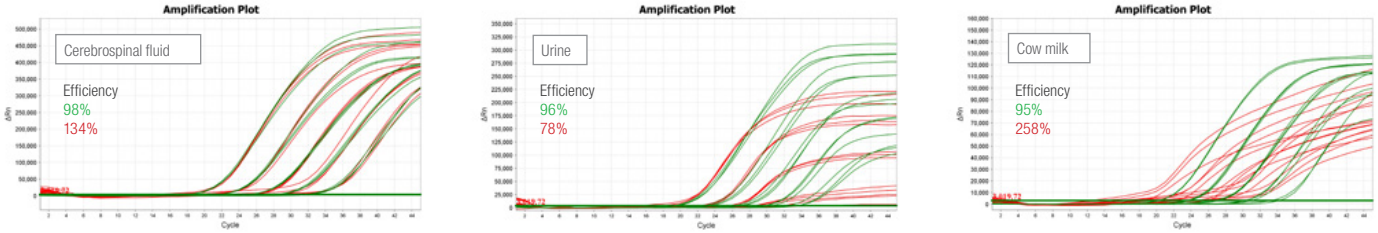


Fig. 2 Efficient amplification from biofluids
A 10-fold serial dilution of genomic DNA was spiked into cerebrospinal fluid, human urine and cow whole milk was amplified, using SensiFAST Probe Direct SuperMix (green) and an Inhibitor-Tolerant Mix from supplier K (red) and the manufacturers' recommended protocol. The results illustrate SensiFAST Probe Direct SuperMix is more sensitive than the mix from supplier K, as lower dilutions could be detected, with better efficiencies for all three inhibitor samples.

Two-Step qPCR Kits

In two-step RT-qPCR, reverse transcription and PCR are performed as two separate reactions, allowing the cDNA and qPCR reactions to be optimized separately, resulting in higher yields of cDNA during the RT step than for one-step procedures, making it more sensitive than one step RT-qPCR.

RNA is converted into cDNA in the first step, using a choice of random hexamers, oligo $d(T)_n$ primers, or gene-specific primers, or for unbiased reverse transcription a mixture of random hexamer and anchored oligo $d(T)_n$ (Such as in the SensiFAST cDNA Synthesis Kit). Either a portion of the RT reaction is diluted into the qPCR in the second step, or the RT reaction is diluted into the qPCR in the second step, or the RT reaction can be extracted and precipitated prior to use, allowing control over the amount of cDNA input. This flexibility is useful when working with genes of variable abundance or on challenging sequences. Any residual DNA cDNA is also available for future amplification reactions of other genes, or even for other applications. The two-step method is particularly useful when the goal is to detect multiple targets from a single sample, or to perform multiple PCR amplifications from a single sample.

- **Reproducible:** consistent results between technical replicates for increased confidence in results
- **Specific:** antibody-mediated hot-start DNA polymerase minimizes non-specific amplification for improved assay sensitivity and reliability
- **Sensitive:** reliable quantification of low abundance targets and scarce samples
- **Robust:** accurate detection of DNA and RNA targets from a broad range of sample types
- **Fast:** delivers reproducible, accurate assay results in as little as 30 minutes

Table 1 Applications for SensiFAST™ kits

Biomarker discovery and validation	Cancer risk assessment	Cellular mRNA and miRNA
ChIP	Detection of extremely low copy targets	DNA damage measurement
Drug therapy efficacy	Gene dosage determination	Gene expression analysis
Gene knockdown validation	Genotyping, allelic discrimination, SNP, haplotyping	High-throughput qPCR
Microarray validation	Microbial quantification	Mitochondrial DNA studies
Pathogen detection	Quantification	Viral load

SensiFAST™ SYBR® Kit

The SensiFAST SYBR® Kit has been developed for fast highly sensitive and reproducible qPCR and has been validated on commonly used in qPCR instruments.

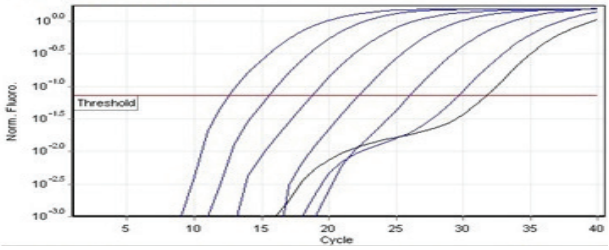
The use of antibodies for the hot-start DNA polymerase system reduces the chances of primer-dimer formation, reducing non-specific priming and leading to greater sensitivity (Fig. 1). The addition of the latest advances in buffer chemistry and enhancers also ensures that the SensiFAST SYBR® Kit produces faster (under 30 minutes), highly reproducible (Fig. 2) qPCR results.

In contrast to specific probes that must be synthesized for each target, SYBR® Green can be used directly in the PCR, making it more convenient and less expensive than probes, however SYBR will detect all double-stranded DNA preventing its use in multiplexing.

> *Very good linearity right down to 10 copies, very good correlation coefficient, good qPCR reaction efficiency and gave a single band on an agarose gel.*
>
> *King's College London, UK*

Product	Size	Cat. #
SensiFAST SYBR No-ROX Kit	500 Reactions	BIO-98005
	2000 Reactions	BIO-98020
	5000 Reactions	BIO-98050
SensiFAST SYBR Lo-ROX Kit	500 Reactions	BIO-94005
	2000 Reactions	BIO-94020
	5000 Reactions	BIO-94050
SensiFAST SYBR Hi-ROX Kit	500 Reactions	BIO-92005
	2000 Reactions	BIO-92020

a) Amplification plot



b) Standard curve

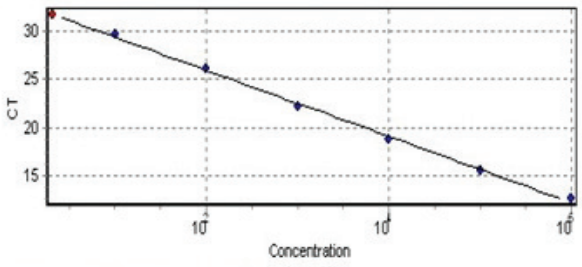


Fig. 1 High sensitivity qPCR under fast cycling conditions.
SensiFAST SYBR No-ROX Kit was used to amplify the rat dopamine 4 receptor using fast cycling conditions (customer results). The process used a 10-fold serial dilution rat DNA (in triplicate) over 7 orders of magnitude. The results illustrate a) very good linearity, down to 10 copies, b) very good correlation coefficient (r2 = 0.998) qPCR reaction efficiency (95%) and c) a single band on an agarose gel (data not shown).

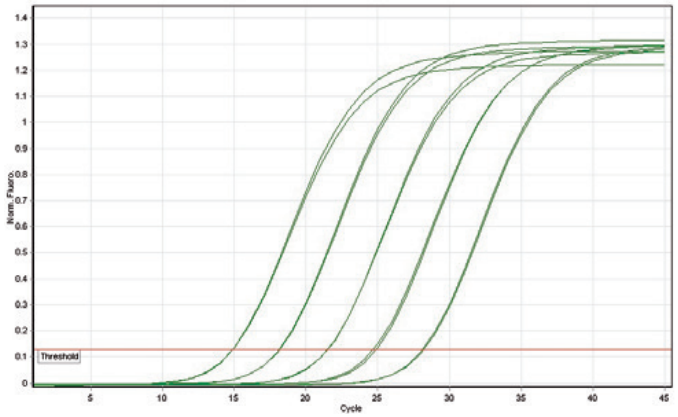


Fig. 2 Reproducibility under fast cycling conditions
The PGK gene diluted in a 10-fold serial dilution of mouse cDNA (in triplicate) over 5 orders of magnitude and amplified using SensiFAST SYBR No-ROX under fast cycling conditions. The results illustrate that the SensiFAST SYBR is fast, highly reproducible and sensitive.

SensiFAST™ Probe Kit

The SensiFAST Probe Kit has been developed for fast qPCR and is designed for superior sensitivity and specificity with probe-detection technology, including TaqMan®, Scorpions® and molecular beacon probes. SensiFAST Probe Kit has been optimized for fast mode on fast qPCR instruments. A combination of the latest advances in buffer chemistry and PCR enhancers, with antibodies for the hot-start DNA polymerase system, ensures that the SensiFAST Probe Kit delivers shorter run times, is highly reproducible, highly-specific and ultra-sensitive (Fig. 1). The advanced buffer chemistry and enhancers also make SensiFAST Probe perfect for multiplexing (Fig. 2), allowing more samples to be run in a day with the highest confidence, ideal for high-throughput assays.

Product	Size	Cat. #
SensiFAST Probe No-ROX Kit	500 Reactions	BIO-86005
	2000 Reactions	BIO-86020
	5000 Reactions	BIO-86050
SensiFAST Probe Lo-ROX Kit	500 Reactions	BIO-84005
	2000 Reactions	BIO-84020
	5000 Reactions	BIO-84050
SensiFAST Probe Hi-ROX Kit	500 Reactions	BIO-82005
	2000 Reactions	BIO-82020

With SensiFAST I was able to dramatically boost my efficiencies and increase reproducibility. In addition, I found that multiple primer pairs produced cleaner products with improved melting curves.

UC Davis School of Veterinary Medicine, USA

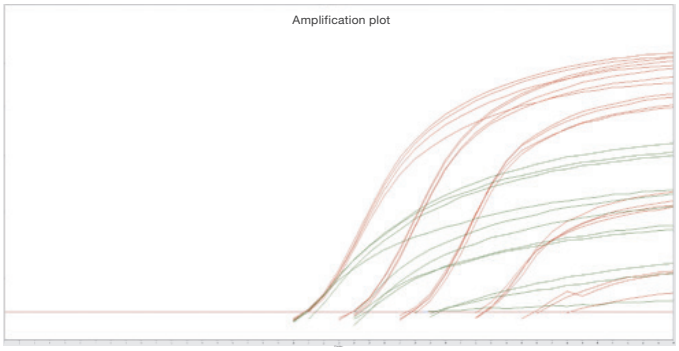


Fig. 1 Sensitivity and reproducibility
Comparison of sensitivity and reproducibility of SensiFAST Probe (red) and a Kit from supplier I (green), the process used a 10-fold serial dilution of human DNA (in quadruplicate) over several orders of magnitude. The results illustrate that SensiFAST Probe is far more sensitive than supplier I.

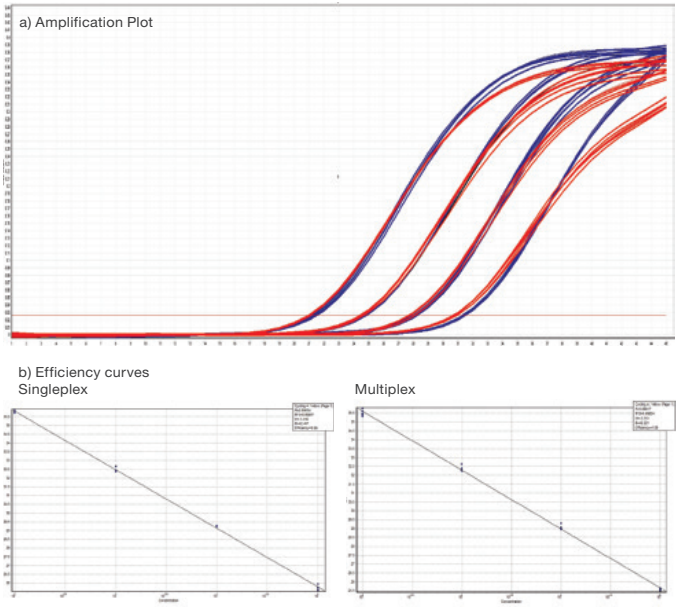


Fig. 2 Sensitivity and efficiency in multiplexing
A 10-fold serial dilution of human cDNA amplified with four different probes; both in singleplex reactions (blue line) and quadruplex reaction (the red line displayed is for the same primers as for the singleplex). Five replicates were run using a conventional TaqMan primer/probe set under fast cycling conditions. SensiFAST Probe No-ROX Kit illustrates a) exactly the same high sensitivity, excellent reproducibility and Ct values for both the singleplex and multiplex reactions and b) no reduction of efficiency that is commonly associated with multiplexing.

SensiFAST™ Lyo-Ready No-ROX Mix

- **Glycerol-free:** ideal for preparation of custom lyophilized qPCR master mixes for improved convenience and extended room-temperature stability
- **Reproducible:** consistent results between technical replicates for increased confidence
- **Robust:** accurate detection of DNA and cDNA targets from a broad range of sample types
- **Sensitive:** reliable quantification of low abundance targets and scarce samples
- **Fast:** delivers reproducible, accurate assay results in as little as 30 minutes

SensiFAST Lyo-Ready No-ROX Mix is a glycerol-free qPCR mix, that can be lyophilized with assay-specific primers and probes to produce ambient temperature stable qPCR master mixes that give outstanding assay reproducibility, sensitivity and robustness following rehydration. The reconstitution of SensiFAST Lyo-Ready No-ROX, containing target-specific primers and probes, with aqueous template is key to reducing handling time and maximizing sample input, while ambient temperature stability of the lyophilized test permits transportation and storage under a range of conditions.

The SensiFAST Lyo-Ready No-ROX Mix delivers the same accurate, highly-reproducible assay performance from the reconstituted mix as it does from the wet mix (Fig. 1) and has been validated in viral detection assays to give the same PCR efficiencies for both singleplex and multiplex for both wet and reconstituted mixes.

Following lyophilization, the SensiFAST Lyo-Ready No-ROX Mix is stable for a minimum of 24 months at room temperature (17 – 23 °C) and for up to three months at 37 °C (Fig. 2). Furthermore, the mix is stable when lyophilized in the absence and presence of primers and probes.

In one-step RT-qPCR format, SensiFAST Lyo-Ready No-ROX Mix can simultaneously detect low abundance RNA and DNA viruses direct from sample (Fig. 3). A wet mix is at two times concentration, so the maximum amount of sample that can be added is an equal volume, however as the volume of SensiFAST Lyo-Ready No-ROX Mix is reduced by lyophilization, larger volumes of sample can be added, thereby increasing the potential sensitivity even further.

Product	Size	Cat. #
SensiFAST Lyo-Ready No-ROX Mix	10 x 10 mL	BIO-11060

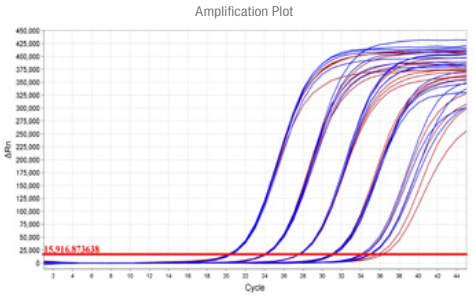


Fig. 1. Sensitivity of wet and lyophilized mixes
The lyophilized (blue) and wet (red) mix amplification profiles for *Actg* amplicons are shown. The results illustrate the same sensitivity and very similar efficiencies, for the wet mix and the lyophilized mix

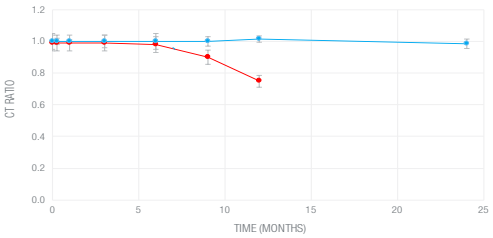


Fig. 2. Storage and stability
Lyophilized SensiFAST Lyo-Ready No-ROX Mix demonstrates stability at room temperature (blue) and 37 °C (red) up to 24 months and 12 months, respectively. Ct values were calculated as a ratio of the lyophilized to wet mix

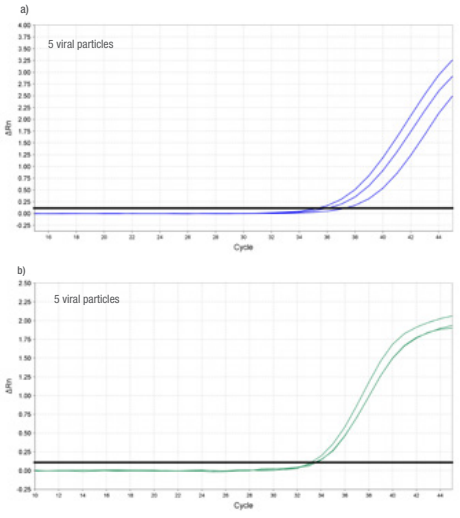


Fig. 3. Simultaneous detection of DNA and RNA viruses
Amplification of viral gene targets in a multiplexed RT-qPCR assay. The amplification profiles show that as few as five viral particles per reaction can be detected for both A. cytomegalovirus (DNA) and B. hepatitis A virus (RNA)

SensiFAST™ HRM Kit

- **Accurate:** clear discrimination of even the most challenging sequence differences
- **Reproducible:** unparalleled consistency between replicate difference plots for increased confidence in sample characterization
- **Sensitive:** efficient detection from even very limiting amounts of sample
- **Flexible:** enables reliable characterization of a broad range of sequence differences
- **Fast:** rapid PCR amplification prior to high resolution melting, enabling higher throughput

SensiFAST HRM Kit incorporates an antibody-mediated hot-start DNA polymerase with EvaGreen® fluorescent dye, to deliver precise, highly reproducible and fast genotyping of sequence variants through high resolution melting.

SensiFAST HRM Kit has been developed for detailed characterization of samples according to their base composition, length and GC content by high resolution melting (HRM). The latest advances in buffer chemistry and enhancers, together with an antibody-mediated hot-start DNA polymerase, ensure SensiFAST HRM Kit delivers reproducible, accurate HRM analysis. SensiFAST HRM Kit enables reliable detection of even single base changes, making it suitable for genotyping single nucleotide polymorphisms (SNPs) (Table 1).

SensiFAST HRM Kit contains EvaGreen®, a third generation saturating fluorescent dye which selectively binds to double-stranded DNA. In contrast to dyes such as SYBR® Green I, EvaGreen can be used at higher concentrations without inhibiting PCR and shows equal binding affinity for GC-rich and AT-rich regions. The combination of SensiFAST DNA Polymerase, a unique buffer system and EvaGreen dye enables amplification and discrimination of even the most challenging sequence differences, such as class 4 SNPs (Fig. 1), without sequence preference. Since it does not require labelled oligonucleotide probes, SensiFAST HRM Kit is also a cost-effective alternative to traditional probe based genotyping methods.

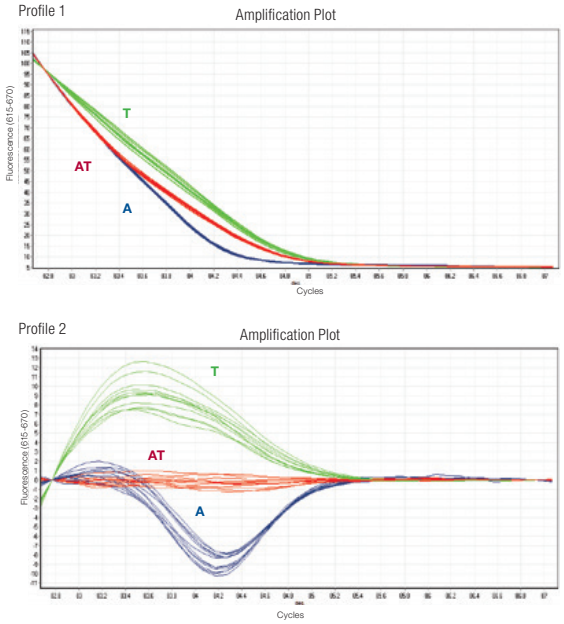


Fig. 1 Highly accurate genotyping of an A/T Class 4 SNP
Three different genotypes of an A>T SNP were analyzed by HRM, using SensiFAST HRM Kit. Normalized HRM melt profiles of each genotype are shown (Profile 1), where A (blue) corresponds to the homozygous wild type, T (green) to the homozygous mutant A/T (red) to heterozygous samples. A difference plot of this data (Profile 2) shows the effect of subtracting an average heterozygous curve from all curves. The results illustrate clear discrimination and highly accurate results even with class 4 SNPs when using SensiFAST HRM.

Table 1. Classes of SNP genotyping

SNP Class	Base Change	Tm Curve Shift	Frequency in humans
1	C/T and G/A	Large > 0.5 ºC	65%
2	C/A and G/T	Large > 0.5 ºC	19%
3	C/G	Small 0.2-0.5 ºC	9%
4	A/T	Small < 0.2 ºC	7%

Product	Size	Cat. #
SensiFAST HRM Kit	500 Reactions	BIO-32005
	2000 Reactions	BIO-32020

SensiFAST™ cDNA Synthesis Kit

- **Efficient:** high-target affinity, coupled with a novel TransAmp™ buffer system for improved yield of full-length cDNA
- **Unbiased:** optimized mix of random hexamers and anchored oligo dT primers for complete 5' to 3' RNA sequence representation
- **Sensitive:** lower Ct values from a broad range of input cDNA concentrations, enabling accurate detection of very low-copy targets
- **Robust:** reliable reverse transcription under challenging conditions, including the use of complex templates and in the presence of inhibitors
- **Fast:** high-yield reverse transcription from a broad range of targets in as little as 5 minutes

To complement the SensiFAST Probe and SYBR® qPCR Kits, Meridian has developed the SensiFAST cDNA Synthesis Kit which displays excellent linearity across a wide range of starting materials. This gives the same relative representation in cDNA templates, regardless of gene abundance, making it excellent for use in qPCR studies.

A novel, highly-pure reverse transcriptase and TransAmp buffer system delivers both highly efficient first strand synthesis (Fig. 1) and higher cDNA yields. This leads to enhanced reproducibility (Fig. 2) and data accuracy. SensiFAST cDNA Synthesis Kit also displays excellent linearity across a wide range of starting material, giving the same relative target representation regardless of input cDNA concentration.

To ensure unbiased 3' and 5' coverage and reverse transcription of all regions in RNA transcripts (Fig. 3), the TransAmp Buffer employs a unique blend of random hexamers and anchored oligo dT primers. Additionally, the SensiFAST cDNA Synthesis Kit can be used with SensiFAST Probe and SYBR Kits for fast qPCR to produce high-quality results in less than 1 hour.

Product	Size	Cat. #
SensiFAST cDNA Synthesis Kit	50 Reactions	BIO-65053
	250 Reactions	BIO-65054

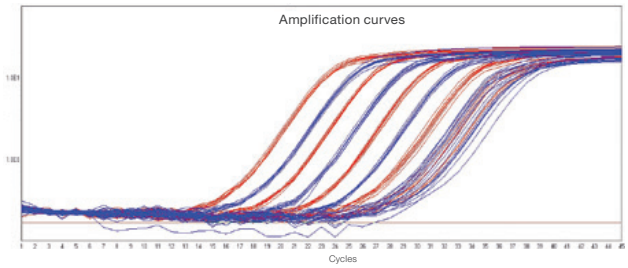


Fig. 1 Speed and sensitivity
SensiFAST cDNA synthesis Kit and a kit from supplier B were used in a first-strand reaction of the same source of total RNA using the manufacturers' recommended conditions. A 10-fold serial dilution was used in a qPCR reaction. The results illustrate that the SensiFAST cDNA Synthesis Kit (red) is also much faster and even more sensitive than supplier B (blue).

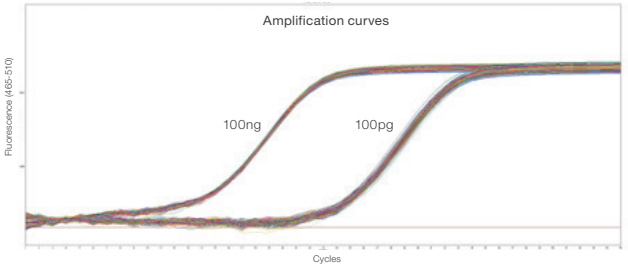


Fig. 2 High reproducibility
SensiFAST cDNA synthesis Kit was employed in 48 independent first-strand reactions, containing 100 ng or 100 pg of total RNA. The first-strand products form the high and input RNA were used in a qPCR assay (reactions performed in triplicate). The results demonstrate the excellent reproducibility of the SensiFAST cDNA Synthesis Kit (the same Ct values), across all 144 wells with 100 ng of input target RNA and all 144 wells with 100 pg of input target RNA.

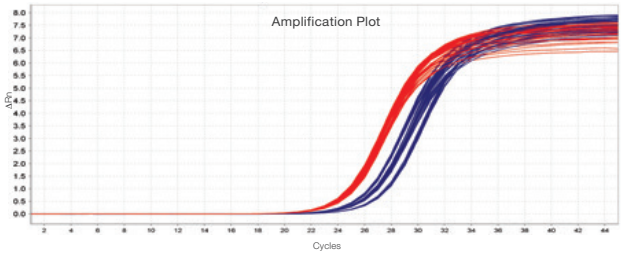


Fig. 3 Unbiased representation across target genes
SensiFAST cDNA Synthesis Kit and a kit from supplier B were used in a first-strand reaction containing total RNA. Primer pairs were designed at 1 kb intervals across the same transcript and used in a qPCR reaction with SensiFAST SYBR® No-ROX. The results illustrate that unlike the results from supplier B (blue). SensiFAST cDNA Synthesis Kit (red) did not show any bias across the intervening transcript.

Ordering Information

SYBR	Size	Cat. #
SensiFAST SYBR® No-ROX One-Step Kit	100 Reactions	BIO-72001
SensiFAST SYBR® No-ROX One-Step Kit	500 Reactions	BIO-72005
SensiFAST SYBR® Hi-ROX One-Step Kit	100 Reactions	BIO-73001
SensiFAST SYBR® Hi-ROX One-Step Kit	500 Reactions	BIO-73005
SensiFAST SYBR® Lo-ROX One-Step Kit	100 Reactions	BIO-74001
SensiFAST SYBR® Lo-ROX One-Step Kit	500 Reactions	BIO-74005
SensiFAST SYBR® No-ROX Kit	500 Reactions	BIO-98005
SensiFAST SYBR® No-ROX Kit	2000 Reactions	BIO-98020
SensiFAST SYBR® No-ROX Kit	5000 Reactions	BIO-98050
SensiFAST SYBR® Hi-ROX Kit	500 Reactions	BIO-92005
SensiFAST SYBR® Hi-ROX Kit	2000 Reactions	BIO-92020
SensiFAST SYBR® Lo-ROX Kit	500 Reactions	BIO-94005
SensiFAST SYBR® Lo-ROX Kit	2000 Reactions	BIO-94020
SensiFAST SYBR® Lo-ROX Kit	5000 Reactions	BIO-94050
SensiFAST SYBR® & Fluorescein Kit	500 Reactions	BIO-96005
SensiFAST SYBR® & Fluorescein Kit	2000 Reactions	BIO-96020

Probe	Size	Cat. #
SensiFAST Probe No-ROX One-Step Kit	100 Reactions	BIO-76001
SensiFAST Probe No-ROX One-Step Kit	500 Reactions	BIO-76005
SensiFAST Probe Hi-ROX One-Step Kit	100 Reactions	BIO-77001
SensiFAST Probe Hi-ROX One-Step Kit	500 Reactions	BIO-77005
SensiFAST Probe Lo-ROX One-Step Kit	100 Reactions	BIO-78001
SensiFAST Probe Lo-ROX One-Step Kit	500 Reactions	BIO-78005
SensiFAST Probe No-ROX Kit	500 Reactions	BIO-86005
SensiFAST Probe No-ROX Kit	2000 Reactions	BIO-86020
SensiFAST Probe No-ROX Kit	5000 Reactions	BIO-86050
SensiFAST Probe Hi-ROX Kit	500 Reactions	BIO-82005
SensiFAST Probe Hi-ROX Kit	2000 Reactions	BIO-82020
SensiFAST Probe Lo-ROX Kit	500 Reactions	BIO-84005
SensiFAST Probe Lo-ROX Kit	2000 Reactions	BIO-84020
SensiFAST Probe Lo-ROX Kit	5000 Reactions	BIO-84050
SensiFAST Probe Direct SuperMix	500 Reactions	BIO-86105
SensiFAST Probe Direct SuperMix	2000 Reactions	BIO-86120

Lyo-Ready	Size	Cat. #
SensiFAST Lyo-Ready No-ROX Mix	10 x 10mL	BIO-11060

cDNA Synthesis	Size	Cat. #
SensiFAST cDNA Synthesis Kit	50 Reactions	BIO-65053
SensiFAST cDNA Synthesis Kit	250 Reactions	BIO-65054

HRM	Size	Cat. #
SensiFAST HRM Kit	500 Reactions	BIO-32005
SensiFAST HRM Kit	2000 Reactions	BIO-32020

Technical Support

For technical assistance or more information on these products, please contact us at mbi.tech@meridianlifescience.com or call us on +49 (0) 3371 60222 03

Global
E: info@meridianlifescience.com
Toll free: +1 800 327 6299

Australia
E: info.au@meridianlifescience.com
Tel: +61 (0)2 9209 4180

meridian BIOSCIENCE®
LIFE DISCOVERED. LIFE DIAGNOSED.