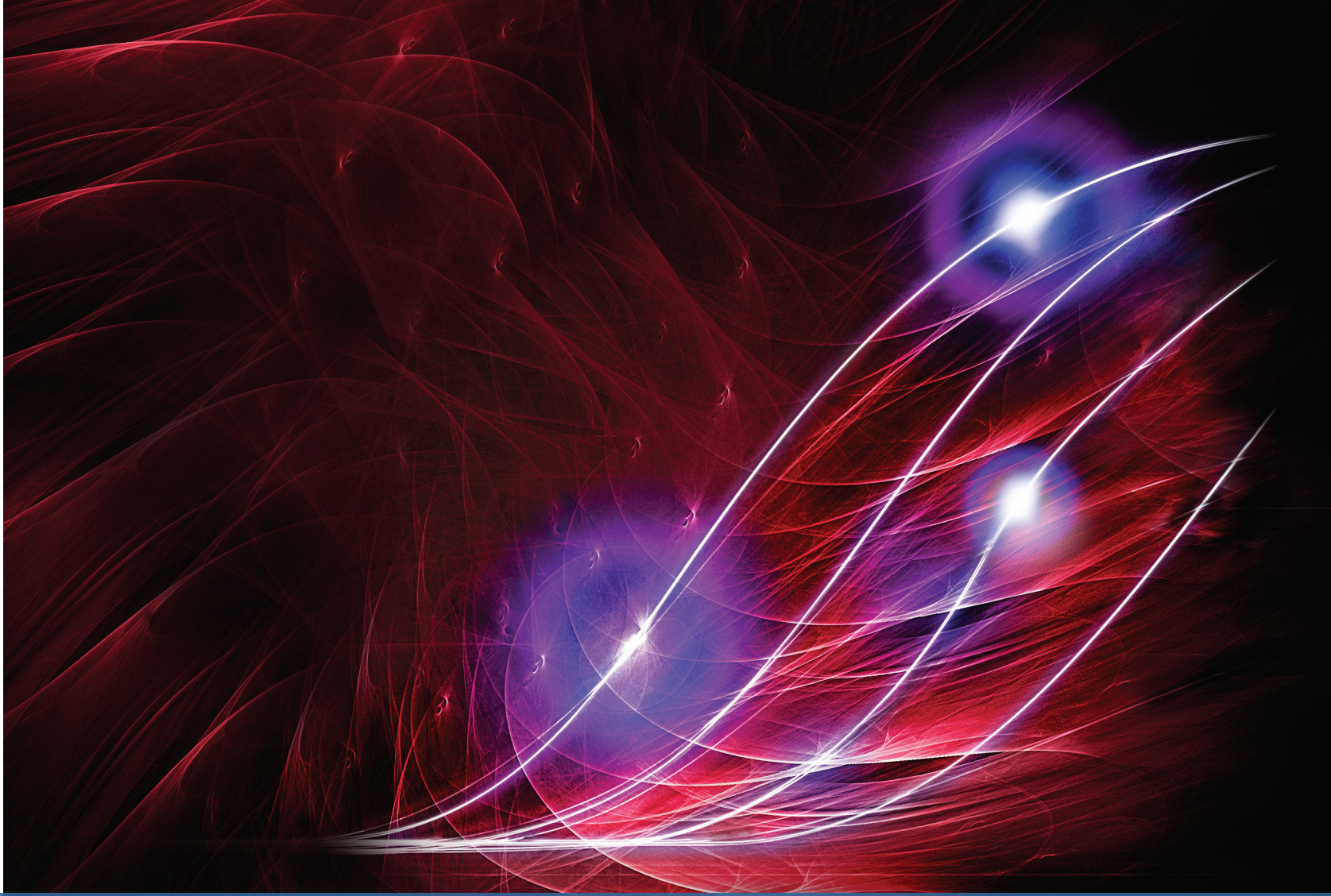

SensiFAST™ Two-Step Real-Time RT-PCR

Superior Fast DNA Analysis

bioline.com/sensifast

BIOLINE

A Meridian Life Science® Company

Two-Step Real-Time RT-PCR

Real-Time PCR is one of the most powerful and sensitive gene analysis techniques available. The main advantage of real-time PCR 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 real-time and can be used in diagnostic, agricultural, biotechnology and pharmaceutical research (see Table 1).

One-step real-time RT-PCR is now the gold standard technique for mRNA detection and quantification. Designed for reverse transcription and real-time PCR amplification in a single tube, it is sensitive enough to enable quantification of RNA from a single cell. SensiFAST™ One-Step Kits are ideal for situations where the same genes must be amplified repetitively, such as in high-throughput detection of a specific target using well-established reaction conditions. However, gene-specific primers are required and all of the cDNA is consumed in the real-time PCR step.

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

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 real-time PCR
Microarray validation	Microbial quantification	Mitochondrial DNA studies
Pathogen detection	Quantification	Viral load

DNA/cDNA Templates			Applied Biosystems™														Cepheid®	PCRmax	Thermo	Takara	Fluidigm	Analytik Jena	Qiagen (Corbett)			Bio-Rad®			Roche			Eppendorf		Techne®		BJS	Agilent (Stratagene)		
			7000	7300	7500	7500 FAST	7700	7900	7900HT	7900HT FAST	StepOne™	StepOne™ plus	ViiA7™	QuantStudio™ 12k Flex	SmartCycler®	Eco™	Piko Real®	Thermal Cycler Dice® (TP800)	BioMark™	qTOWER/qTOWER 2	Rotor-Gene™ 3000	Rotor-Gene™ 6000	Rotor-Gene™ Q	iCycler®	MyiQ™	iQ™5	Opticon™	Opticon™2	Chromo4™	MiniOpticon™	CFX96™	CFX384™	LightCycler® 96	LightCycler® 480	LightCycler® Nano	Mastercycler® ep realplex	Mastercycler® ep realplex 2S	Quantica®	PrimeQ	Xxpress®	MX4000P®	MX3000P®	MX3005P®
SYBR Green Assays	SensiFAST™ SYBR No-ROX Kit														✓	✓	✓	✓																								
	SensiFAST™ SYBR Hi-ROX Kit		✓	✓			✓	✓	✓	✓	✓	✓																												
	SensiFAST™ SYBR Lo-ROX Kit				✓	✓							✓	✓					✓																						
	SensiFAST™ SYBR & Fluorescein Kit																							✓	✓	✓														
Probe Assays	SensiFAST™ Probe No-ROX Kit*														✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	SensiFAST™ Probe Hi-ROX Kit		✓	✓			✓	✓	✓	✓	✓	✓																												
	SensiFAST™ Probe Lo-ROX Kit				✓	✓							✓	✓					✓																						
	SensiFAST™ Hi-ROX Genotyping Kit		✓	✓			✓	✓	✓	✓	✓	✓																												
	SensiFAST™ Lo-ROX Genotyping Kit				✓	✓							✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
HRM	SensiFAST™ HRM Kit					✓				✓			✓	✓		✓						✓	✓								✓	✓		✓		✓	✓				

For a full listing of instrument compatibility visit bioline.com/sensifast

SensiFAST™ cDNA Synthesis Kit

- Reproducible: unique reverse transcriptase and TransAmp™ buffer generate consistent first-strand cDNA
- Broad dynamic range: ideal for dilute and low-copy samples
- Sensitive: real-time RT-PCR analysis from as little as 1pg of starting total RNA
- Unbiased cDNA synthesis: complete 5' to 3' RNA sequence representation

To complement the SensiFAST™ Probe and SYBR® kits, Bioline has developed the SensiFAST™ cDNA Synthesis Kit to provide a rapid and sensitive method for first-strand cDNA synthesis. The SensiFAST™ cDNA Synthesis Kit displays excellent linearity across a wide range of starting material revealing the same relative representation in cDNA templates, regardless of gene abundance, making it excellent for use in real-time PCR studies.

A novel, highly-pure reverse transcriptase and new TransAmp™ buffer system delivers highly-efficient synthesis of cDNA (Fig. 1), enhanced reproducibility (Fig. 2) and data accuracy. These features make the SensiFAST™ cDNA Synthesis Kit ideal for working with limited samples, such as laser-microdissected samples and tissue biopsies. The TransAmp Buffer also employs a unique blend of random hexamer primers and anchored oligo dT to ensure unbiased 3' and 5' coverage and reverse transcription of all regions (Fig. 3). Additionally, the SensiFAST™ cDNA Synthesis Kit can be used with SensiFAST™ Probe and SYBR® Kits for fast real-time RT-PCR without compromising quality, with real-time results in less than an hour.

Sensitivity and Speed

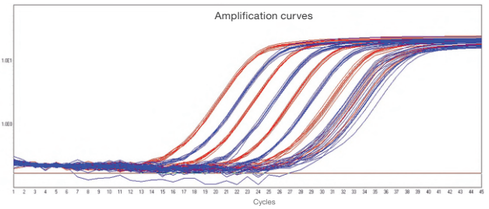

Fig. 1 Comparison of the SensiFAST™ cDNA Synthesis
The SensiFAST cDNA Synthesis Kit and a kit from supplier B were used in a first-strand reaction of the same source of total RNA using the manufacturer's recommended conditions. A 10-fold serial dilution was used in a real-time PCR reaction. The results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

Reproducibility

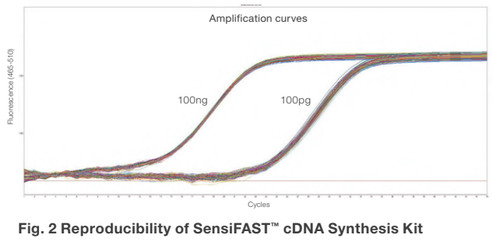

Fig. 2 Reproducibility of SensiFAST™ cDNA Synthesis Kit
The SensiFAST cDNA Synthesis Kit was employed in 48 independent first-strand reactions, containing 100ng or 100pg of total RNA. The first-strand products from the high and low input RNA were used in a real-time PCR assay (reactions performed in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

Unbiased representation

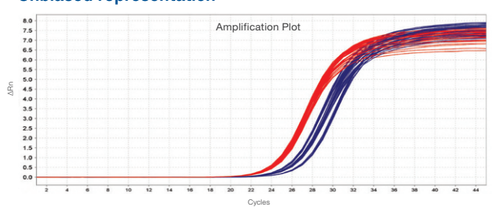

Fig. 3 Unbiased representation across target genes
The SensiFAST cDNA Synthesis Kit and a kit from supplier B were used in a first-strand reaction containing total RNA. Primer pairs were designed at 1kb intervals across the same transcript and used in a real-time PCR reaction with SensiFAST SYBR® No-ROX.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

SensiFAST™ SYBR® Kit

- Accurate quantification: hot-start capability reduces primer-dimer formation
- Sensitive: from low copy targets
- Rapid: unique buffer chemistry for highest specificity and sensitivity and saves time
- Flexible: compatible with all standard and fast cycling instruments

The SensiFAST™ SYBR® Kit has been developed for fast, highly sensitive and reproducible real-time PCR and has been validated on commonly used real-time instruments. The use of antibodies for the hot-start DNA polymerase system reduces the chances of primer-dimer formation, reducing nonspecific 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) real-time PCR 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 multiplexing.

Sensitivity

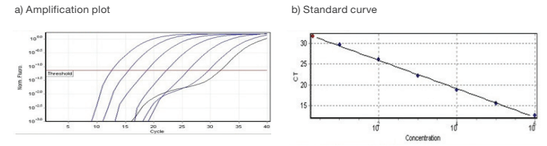

Fig. 1 SensiFAST™ SYBR® No-ROX used to amplify the rat dopamine 4 receptor using fast cycling conditions (customer results).
The process used a 10-fold serial dilution rat DNA (in triplicate) over 7 orders of magnitude. The results illustrate a) very good linearity right down to 10 copies and b) very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

Speed

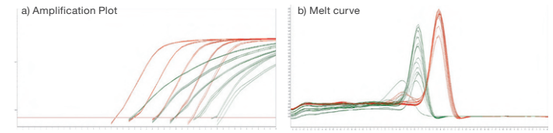

Fig. 2 Comparison of SensiFAST™ SYBR® Hi-ROX (red) against supplier Q (green) using fast cycling conditions.
The process used a 10-fold serial dilution of human cDNA (in quadruplicate) over 4 orders of magnitude. The conditions were 95°C for 2min and 45 cycles of 95°C 10s, 60°C 15s. The results illustrate that the SensiFAST™ SYBR® Hi-ROX was faster (earlier Ct) with higher reproducibility, particularly at lower concentrations.

> *"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

* Dr David Sugden, School of Medicine, King's College London. UK

SensiFAST™ Probe Kit

- Highly specific: minimal non-specific activity leading to better efficiency
- Ultra-sensitive: perfect for low copy number samples
- Fast: unique buffer chemistry allowing fast protocols and higher productivity
- Efficient multiplexing: no loss in efficiency using multiple probes

The SensiFAST™ Probe Kit has also been developed for fast real-time PCR and is designed for superior sensitivity and specificity with probe-detection technology, including TaqMan®, Scorpions® and molecular beacon probes. The SensiFAST™ Probe Kit has been optimized for fast mode on fast real-time PCR instruments and fast cycling conditions on standard real-time PCR 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.

"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

Sensitivity and reproducibility

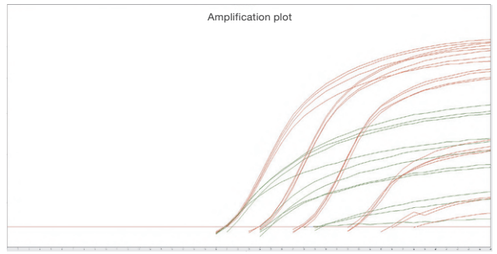

Fig. 1 Comparison of sensitivity and reproducibility of SensiFAST™ Probe (red) and a kit from supplier I (green)
The process used a 10-fold serial dilution of human DNA (in quadruplicate) over several orders of magnitude. The conditions were 95°C for 2min and 45 cycles of 95°C 10s, 60°C 15s. The results illustrate that SensiFAST™ Probe is far more sensitive than supplier I.

Sensitivity and efficiency in multiplexing

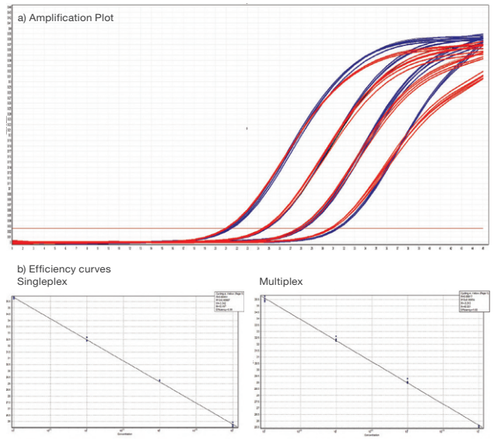

Fig. 2 Comparison of sensitivity and efficiency in a singleplex and quadruplex reaction
The process used a 10-fold serial dilution of human cDNA amplified with four different probes; both in singleplex reactions (blue line) and a quadruplex reaction (the red line displayed is for the same primers as for the singleplex). Five replicates were run using a conventional TaqMan primer/probe set under fast cycling conditions (3 min 95°C followed by 45 cycles 95°C 10s, 60°C 10s). SensiFAST™ Probe No-ROX 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™ Genotyping Kit

- Accurate allele calling: clear allele discrimination for higher confidence genotype calling
- Tight fluorescence clusters: demonstrate high reproducibility and are perfect for difficult SNPs
- Faster run time: enabling more experiments per day, ideal for large genotyping projects
- Designed for dual-labeled probe assays: for example TaqMan® probe-based assays

The SensiFAST™ Genotyping Kit has been developed for fast, precise and highly reproducible genotyping of sequence variants, including loci with type IV SNPs. The SensiFAST™ Genotyping Kit is a combination of the latest advances in buffer chemistry, together with antibodies for the hot-start DNA polymerase system. This ensures that the SensiFAST™ Genotyping Kit produces highly-specific, ultra-sensitive real-time PCR with clear allelic discrimination and outstanding allele clustering (Fig. 1). The SensiFAST™ Genotyping Kit is therefore ideal for high throughput with many dual-labeled probe assays (including TaqMan® probe based assays) from a wide range of sources, including human, animal and plant samples.

The advanced buffer chemistry in the SensiFAST™ Genotyping Kit provides higher stringency and more specific binding of the allele-specific probes, resulting in much narrower probe melting temperature windows. The combination of these developments leads to wider and clearer separation of allele clusters. The kit allows robust genotype calling enabling reproducible and accurate results. The advanced buffer also means that minimal optimization is required to achieve fast, reproducible and accurate results, ideal for high throughput screening.

Allelic clustering and discrimination

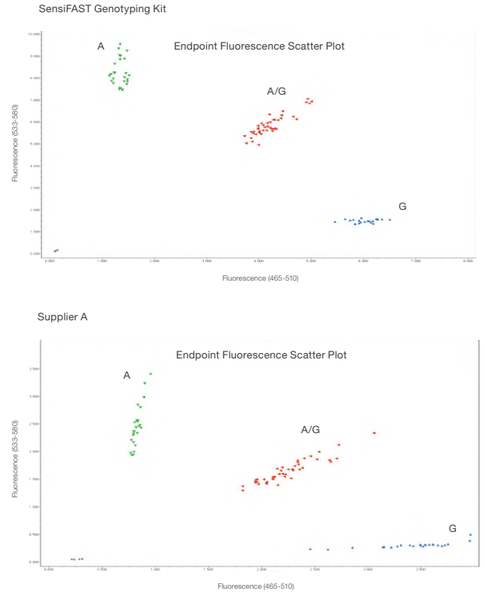

Fig. 1 Allele clustering
DNA samples from ninety six patients were genotyped using the SensiFAST Genotyping kit and a kit from supplier A. The results illustrate the SensiFAST Genotyping Kit gives defined clusters while Supplier A gives poorly defined trailing clusters and low confidence calling.

Ordering Information

SYBR®	Size	Cat. #
SensiFAST™ SYBR® No-ROX Kit	200 Reactions	BIO-98002
SensiFAST™ SYBR® No-ROX Kit	500 Reactions	BIO-98005
SensiFAST™ SYBR® No-ROX Kit	2000 Reactions	BIO-98020
SensiFAST™ SYBR® Hi-ROX Kit	200 Reactions	BIO-92002
SensiFAST™ SYBR® Hi-ROX Kit	500 Reactions	BIO-92005
SensiFAST™ SYBR® Hi-ROX Kit	2000 Reactions	BIO-92020
SensiFAST™ SYBR® Lo-ROX Kit	200 Reactions	BIO-94002
SensiFAST™ SYBR® Lo-ROX Kit	500 Reactions	BIO-94005
SensiFAST™ SYBR® Lo-ROX Kit	2000 Reactions	BIO-94020
SensiFAST™ SYBR® & Fluorescein Kit	200 Reactions	BIO-96002
SensiFAST™ SYBR® & Fluorescein Kit	500 Reactions	BIO-96005
SensiFAST™ SYBR® & Fluorescein Kit	2000 Reactions	BIO-96020

Probe	Size	Cat. #
SensiFAST™ Probe No-ROX Kit	200 Reactions	BIO-86002
SensiFAST™ Probe No-ROX Kit	500 Reactions	BIO-86005
SensiFAST™ Probe No-ROX Kit	2000 Reactions	BIO-86020
SensiFAST™ Probe Hi-ROX Kit	200 Reactions	BIO-82002
SensiFAST™ Probe Hi-ROX Kit	500 Reactions	BIO-82005
SensiFAST™ Probe Hi-ROX Kit	2000 Reactions	BIO-82020
SensiFAST™ Probe Lo-ROX Kit	200 Reactions	BIO-84002
SensiFAST™ Probe Lo-ROX Kit	500 Reactions	BIO-84005
SensiFAST™ Probe Lo-ROX Kit	2000 Reactions	BIO-84020

Genotyping	Size	Cat. #
SensiFAST™ Hi-ROX Genotyping Kit	200 Reactions	BIO-35002
SensiFAST™ Hi-ROX Genotyping Kit	500 Reactions	BIO-35005
SensiFAST™ Hi-ROX Genotyping Kit	2000 Reactions	BIO-35020
SensiFAST™ Lo-ROX Genotyping Kit	200 Reactions	BIO-34002
SensiFAST™ Lo-ROX Genotyping Kit	500 Reactions	BIO-34005
SensiFAST™ Lo-ROX Genotyping Kit	2000 Reactions	BIO-34020

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

Please contact us for institutional pricing, special price quotations and availability of bulk pack sizes.

PGGBL0715V1.2

BIOLINE

A Meridian Life Science® Company

Bioline USA Inc.
email: info.us@bioline.com
Order Toll Free: +1 888 257 5155

Bioline Reagents Ltd
email: info.uk@bioline.com
Tel: +44 (0)20 8830 5300

Bioline (Aust) Pty Ltd
email: info.au@bioline.com
Tel: +61 (0)2 9209 4180

Bioline GmbH
email: info.de@bioline.com
Tel: +49 (0)3371 68 12 29

Meridian Bioscience Asia Pte. Ltd.
email: info.sg@bioline.com
Toll Free: 1800 BIOLINE (2465463)

Bioline France
email: info.fr@bioline.com
Tel: +33 (0)1 42 56 04 40