SensiFAST™ SYBR® Kits

Superior Fast Gene Expression Analysis

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Des femmes et des hommes
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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

a) Amplification plot

b) Standard curve

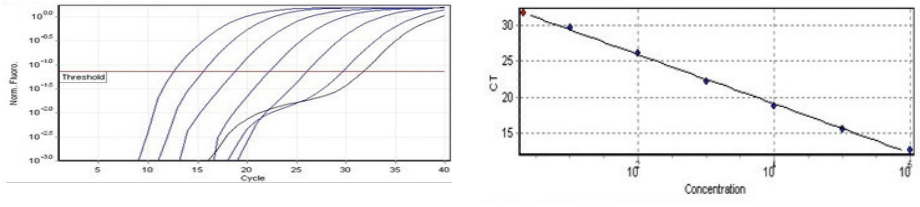

Fig. 1 High sensitivity qPCR under fast cycling conditions.
SensiFAST SYBR No-ROX Kit was used to amplify the rat dopamine 4 receptor using fast cycling conditions (customer results). The process used a 10-fold serial dilution rat DNA (in triplicate) over 7 orders of magnitude. The results illustrate 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).

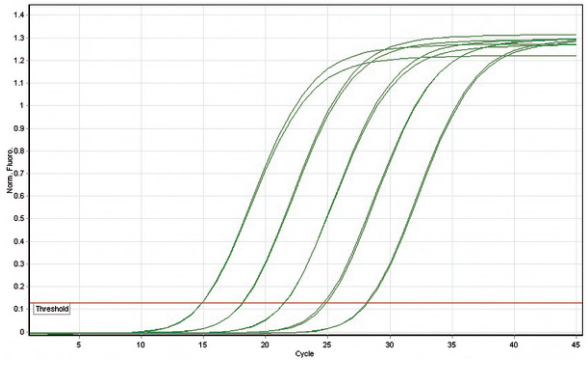

Fig. 2 Reproducibility under fast cycling conditions
The PGK gene diluted in a 10-fold serial dilution of mouse cDNA (in triplicate) over 5 orders of magnitude and amplified using SensiFAST SYBR No-ROX under fast cycling conditions. The results illustrate that the SensiFAST SYBR is fast, highly reproducible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

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

SensiFAST Selection Table

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

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