

SensiFAST[™] One-Step Real-Time RT-PCR

Superior Fast Gene Expression Analysis





- Accurate quantification: efficient one-step, one-tube RNA quantification, reducing experimental variation
- Sensitive: enabling low copy number detection from RNA samples
- Fast: optimized proprietary mixture of enzymes and optimized RT buffer chemistry
- Flexible: consistent performance with a wide variety of RNA targets, including complex viral RNA
- Reliable: for performing analysis of single targets from multiple RNA samples

SensiFAST™ One-Step Kits are a complete range of highly-optimized ready-to-use kits, designed for the reverse transcription and real-time PCR amplification of a specific target RNA, from either total RNA or mRNA, in a single tube. This reduces the number of sample manipulations required and time-to-result. In contrast to two-step strategies, gene-specific primers are required and all of the cDNA is consumed in the real-time PCR step.

The kits use the latest developments in real-time reverse transcription PCR (real-time RT-PCR) to realize the fastest cycling times and greatest sensitivity, without compromising accuracy, reproducibility or performance. SensiFAST™ One-Step Kits can be used on all real-time instruments and are perfect for the new generation of fast PCR cyclers.

SensiFAST™ One-Step Kits provide the perfect solution when processing many samples at a time, for one or a few targets per sample. The kits can be used in assays such as virus detection and quantification, and high-throughput gene expression screening without compromising sensitivity and reproducibility (see Table 1).

Exceed the limit with SensiFAST[™] One-Step Kits, available in a variety of configurations to suit all of your different applications and techniques.



Table 1 Applications for SensiFAST™ kits

Table I Applications	IUI SEIISIFAST KILS	
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

			Applied Biosystems***	7000 7300	7500 7500 EAST	7700	7900	7900HT	7900HT FAST StepOne™	StepOne" plus	ViiA7™ OuantStudio™ 12k Flex	SmartCycler® PCBmax	Eco™	Thermo Piko Real®	Takara	Thermal Cycler Dice® (TP800)	Fluidigm BioMark [™]	Analytik Jena qTOWER/qTOWER 2	Qiagen (Corbett)	Rotor-Gene™ 3000	Rotor-Gene™ 6000	iCycler® Mvin™	inying iQ™5	0pticon™	Opticon"2	Girono4 MiniOpticon™	CFX96™	CFX384™	LightCycler® 96	@ @	Eppendorf	@	Mastercycler® ep realplex 2S Techne®	Quantica®	PrimeQ B IS	Xxpress®	Agilent (Stratagene)	MX4000P® MX3000P®	MX3005P®
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 * Used for all instruments when multiplexing

SensiFAST[™] SYBR[®] One-Step Kit

The SensiFAST™ SYBR® One-Step Kit has been developed for fast, highly sensitive real-time RT-PCR and has been validated on all commonly used real-time instruments. The unique formulation has been designed for highly reproducible first-strand cDNA synthesis and subsequent real-time PCR in a single tube. The use of high-affinity antibodies for the hot-start DNA polymerase system reduces the chances of primer-dimer formation, allowing a much greater dynamic range by removing competition for reaction components during amplification, leading to greater sensitivity (Fig. 1) and reproducibility (Fig. 2) than competitor kits. The addition of the latest advances in buffer chemistry and enhancers also ensure that the SensiFAST™ SYBR® One-Step Kit produces fast, highly specific one-step real-time RT-PCR.

"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

Sensitivity and specificity

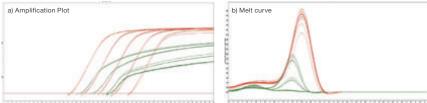


Fig. 1 Comparison of SensiFAST™ SYBR® Hi-ROX One-Step (red) against supplier Q (green) using fast cycling conditions

A 10-fold serial dilution of human RNA (in triplicate) over 5 orders of magnitude. The conditions were 45°C 10min followed by 95°C for 5min and 35 cycles of 95°C 10s, 60°C 10s and 72°C 5s. The results illustrate that SensiFAST™ SYBR® One-Step was faster (earlier Ct) and more sensitive, without the primer-dimers seen with other suppliers.

Reproducibility

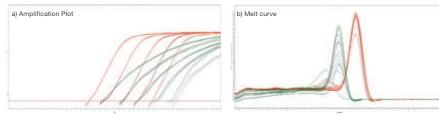


Fig. 2 Comparison of SensiFAST™ SYBR® Lo-ROX One-Step (red) against supplier A (green) using fast cycling conditions

A 10-fold serial dilution of human RNA (in triplicate) over 5 orders of magnitude. The conditions were 45°C 10min followed by 95°C for 5min and 35 cycles of 95°C 10s, 60°C 10s and 72°C 5s. The earlier Ct values illustrate that SensiFAST™ SYBR® One-Step Kit is faster and the tight replicates illustrate that the SensiFAST™ SYBR® One-Step Kit delivers more reproducibility.

SensiFAST[™] Probe One-Step Kit

The SensiFAST[™] Probe One-Step Kit has been developed for fast real-time RT-PCR and is designed for superior sensitivity and specificity with probe-detection technology, including TaqMan®, Scorpions® and molecular beacon probes. The SensiFAST[™] Probe One-Step Kits have been formulated for highly reproducible first-strand cDNA synthesis and subsequent real-time PCR in a single tube. The latest advances in buffer chemistry and enhancers, together with high-affinity antibodies ensure fast, ultra-sensitive (Fig. 3) and highly-specific one-step real-time RT-PCR (Fig. 4). This also gives the SensiFAST[™] Probe One-Step Kit unmatched efficiency in multiplexing (Fig. 5).

Sensitivity

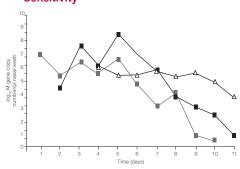


Fig. 3 Copy number of viral RNA in nasal washes The WHO Collaborating Centre for Reference and Research on Influenza in Australia used real-time RT-PCR analysis of a mixed population of influenza viruses in ferret nasal washes to measure the viral replication and transmission kinetics of each virus population (*Butler et al 2014). The copy number of viral RNA in each nasal wash was determined over 11 days using the SensiFAST Probe Lo-ROX One-Step Kit. The results show sensitivity of real-time RT-PCR, helping to show the fitness advantage conferred by mutations in drug-resistant influenza viruses.

Multiplexing

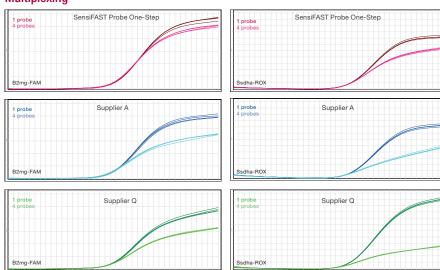


Fig. 5 Comparison of SensiFAST SYBR® Hi-ROX One-Step (red), suppliers A (blue) and Q (green) A 100-fold dilution of human cDNA was used with four probes, either in singleplex reactions (darker line) or in quadruplex reaction (lighter line), using a conventional TaqMan primer/probe set. The results illustrate that the SensiFAST Probe One-Step Kit is very efficient in delivering the same Ct in singleplex and multiplex assays.

One-Step Real-Time RT-PCR

"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 log₁₀ dynamic range"

University of Queensland, Australia*

Specificity

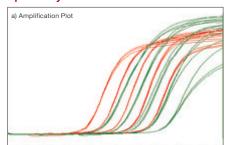


Fig. 4 Comparison of SensiFAST™ Probe One-Step (red) against supplier A (green) using fast cycling conditions

Mouse β-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 much faster by four Ct (more than 10-fold).

^{*} Wang C.Y.T., et al A novel duplex real-time PCR for HPIV-4 detects co-circulation of both viral subtypes among ill children during 2008 *J. Clinical Virology* **54(1)**: 83-85 (2012)

^{*} Butler, J., et al (2014). Estimating the Fitness Advantage Conferred by Permissive Neuraminidase Mutations in Recent Oseltamivir-Resistant A (H1N1) pdm09 Influenza Viruses. PLoS pathogens, 10(4), e1004065.

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® & Fluorescein One-Step Kit	100 Reactions	BIO-75001
SensiFAST™ SYBR® & Fluorescein One-Step Kit	500 Reactions	BIO-75005
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
	500 Reactions	BIO-78005

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

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