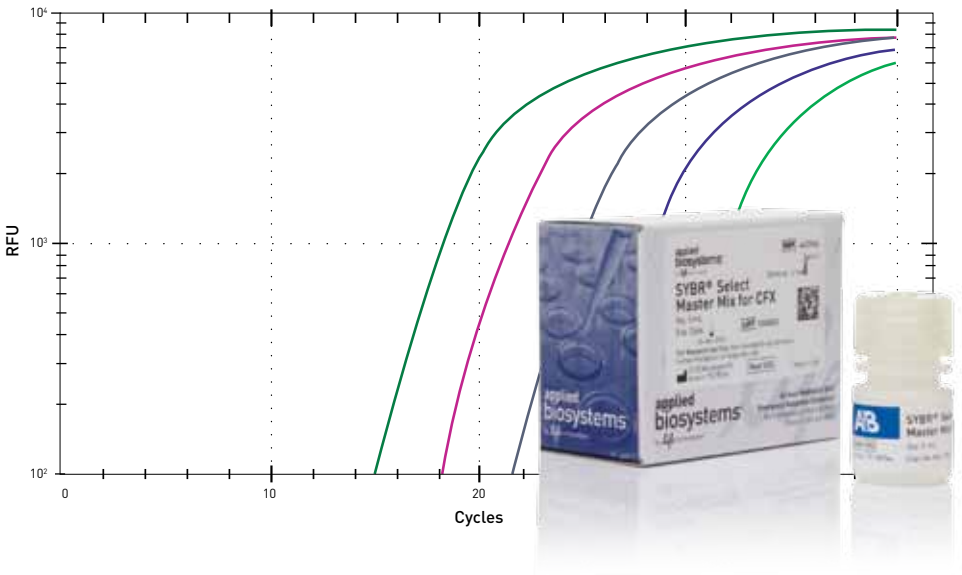
applied biosystems®
by life technologies™

SYBR® Select Master Mix for CFX

for highly specific and sensitive quantitation

SYBR® Select Master Mix for CFX offers advanced performance at an affordable price. SYBR® Select Master Mix for CFX is formulated to provide superior specificity and reproducibility without compromising sensitivity and dynamic range in your real-time quantitative PCR (qPCR) experiments.

SYBR® Select Master Mix for CFX delivers highly specific and sensitive DNA, cDNA, and RNA quantitation, with true single-copy detection. SYBR® Select Master Mix for CFX offers significantly improved specificity by employing highly purified AmpliTaq® DNA Polymerase, UP (Ultra Pure), which is activated by a proprietary hot-start mechanism. SYBR® Select Master Mix for CFX is formulated with SYBR® GreenER™ dye, which provides both brighter signal and lower PCR inhibition than SYBR® Green I dye. In addition, SYBR® Select Master Mix for CFX contains heat-labile uracil-DNA glycosylase (UDG) for worry-free carryover contamination control.



Benefits

- Superior specificity and reproducibility
- Brighter signals
- Stability for 72 hours before and after qPCR
- True single-copy detection
- Compatible with the Bio-Rad® CFX96™ Touch and CFX384™ Touch Real-Time PCR Detection Systems in standard cycling mode

life technologies™

Optimized formulation for superior performance

SYBR® Select Master Mix for CFX contains all components, excluding the template and primers, in a convenient 2X mix for highly specific and sensitive real-time PCR reactions. Components of the master mix in the optimized buffer include:

- AmpliTaq® Polymerase, UP, a highly purified DNA polymerase engineered with a proprietary hot-start mechanism that provides exceptional specificity and pre-PCR benchtop stability
- SYBR® GreenER™ dye to detect double-stranded DNA—SYBR® GreenER™ dyes is brighter and less inhibitory to PCR than SYBR® Green I dye
- Heat-labile uracil-DNA glycosylase (UDG) and a dNTP blend of dUTP/dTTP are included to maintain optimal PCR results and permits worry-free carryover contamination control

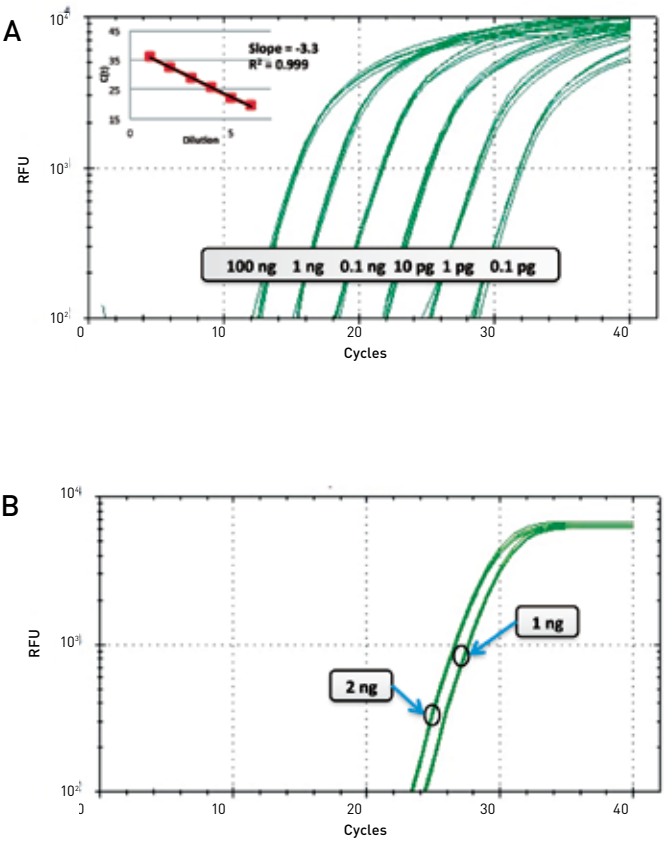


Figure 1. Use a wide range of input cDNA and obtain excellent linearity and discrimination. (A) Amplification plots and dilution curve (inset) for PGK1 amplified from human cDNA using the Bio-Rad® CFX384™ Touch Real-Time PCR instrument. Up to 100 ng of input cDNA can be used without PCR inhibition or diminished PCR efficiency. **(B)** Discrimination is achieved for 1 ng vs. 2 ng cDNA input.

Reproducible results across a wide dynamic range

SYBR® Select Master Mix for CFX is able to accommodate a wide range of input cDNA without compromising PCR efficiency or reproducibility. The *PGK1* gene was amplified from a 10-fold dilution series of human cDNA, using the Bio-Rad® CFX384™ Touch Real-Time PCR Detection System, to demonstrate the superior range and reliability of the SYBR® Select Master Mix for CFX. The amplification plot and standard curve show that the SYBR® Select Master Mix for CFX can be used with as much as 100 ng cDNA per reaction without PCR inhibition or diminished PCR efficiency (Figure 1A). Figure 1B demonstrates the excellent 2-fold discrimination achieved using SYBR® Select Master Mix for CFX, as shown by comparing the amplification curves of 1 ng and 2 ng cDNA input with the same *PGK1* target.

Formulated to ensure maximum specificity

SYBR® Select Master Mix for CFX was tested with 33 assays representing a broad range of amplicon lengths (48–145 bp) and GC content (37%–75%). Melt curves were generated after amplification to determine the specificity of the reactions. Single T_m peaks were obtained for all 33 assays, demonstrating that 100% of the assays generated a single amplicon without any primer-dimer or nonspecific amplification. Representative melt curves are shown in Figure 2.

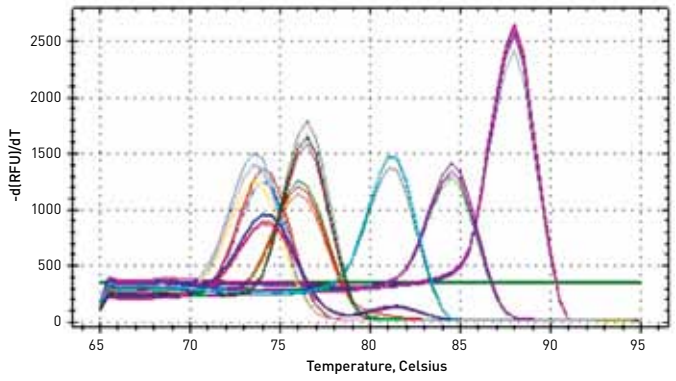


Figure 2. Representative melt curves for target genes amplified from human cDNA. Eight melt curves, with 4 replicates of each, are shown for *A2M*, *ACACA*, *AOF1*, *APOA1*, *ARL1*, β-actin, *BMP2*, and *CCNB1* assays.

72 hours of pre- and post-PCR benchtop stability

Pre-PCR benchtop stability was tested with 22 assays and 2 ng of human cDNA per well. Three replicate plates were set up in parallel with SYBR® Select Master Mix for CFX, primers, and samples. Real-time PCR was performed immediately after preparation, and after 24 and 72 hours at room temperature and exposure to ambient light. While a slight decrease in fluorescent signal is observed (approximately 20% decrease), reactions containing a premix of SYBR® Select Master Mix for CFX, primers, and sample are stable for up to 72 hours (Figure 3). Melt curves obtained 72 hours after amplification demonstrate that the PCR products are sufficiently stable to enable post-PCR analysis several days after amplification (data not shown).

True single-copy detection using digital PCR

Digital PCR works by dividing a sample into many individual real-time PCR reactions so that only some of the reactions contain the target. After amplification, the number of wells containing amplified material is counted to obtain an absolute answer for the exact number of copies in the sample. Digital PCR is reliable only if the real-time PCR reaction can reliably detect single copies of target.

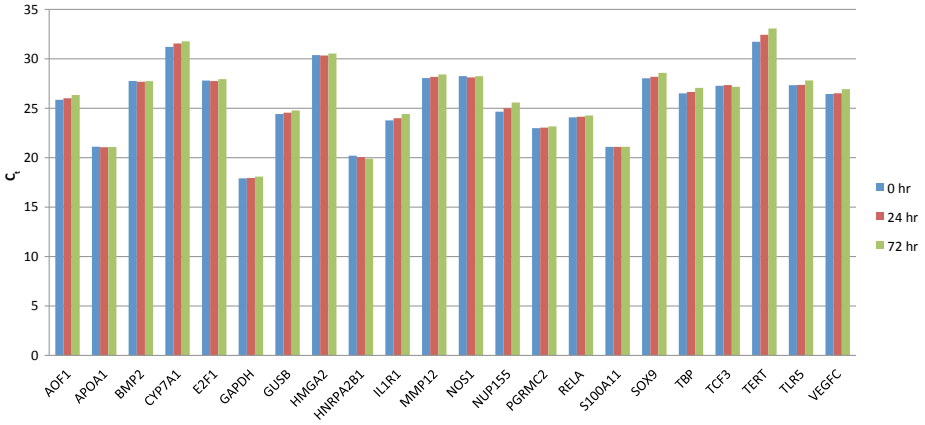


Figure 3. Pre-PCR benchtop stability. Three replicate plates were set up in parallel with SYBR® Select Master Mix for CFX, primers, and samples. Real-time PCR was performed immediately and at 24 and 72 hours after preparation. C_t values for 22 assays are shown.

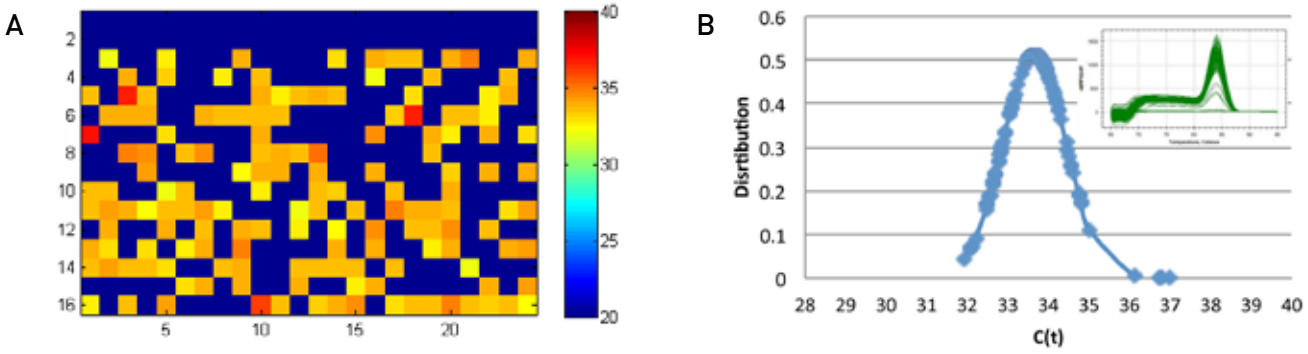


Figure 4. Single-copy detection using digital qPCR with the CFX384™ Touch Real-Time PCR Detection System. (A) Digital PCR heat map. Genomic DNA was diluted to <1 copy per reaction to ensure that most wells would receive 1 or 0 copies of DNA. **(B)** A normal distribution of the resulting C_t values was obtained after amplification of the single-copy RNase P gene. The melt curves of all the amplified material produced a single peak (inset).

The single-copy RNase P gene was amplified using genomic DNA diluted to less than 1 copy per reaction to ensure that most wells would receive 1 or 0 copies of DNA. Approximately 50% of the wells did not have amplified DNA, as shown in Figure 4A. Single-copy detection using SYBR® Select Master Mix for CFX was achieved, as demonstrated by the normal distribution of the C_t values and the specificity of the reactions (Figure 4B).

Carryover contamination control with heat-labile UDG

SYBR® Select Master Mix for CFX contains a heat-labile uracil-DNA glycosylase (UDG) that degrades previously amplified dUTP-containing DNA to help reduce carryover contamination. A spike-in experiment with previously amplified DNA demonstrated that the heat-labile UDG decreased the concentration of dUTP-containing DNA 100-fold (data not shown). With standard UDG, PCR products are degraded soon after amplification due to residual UDG activity. With the heat-labile UDG used in the SYBR® Select Master Mix for CFX, PCR products are stable for up to 72 hour after amplification (data not shown), demonstrating that the heat-labile UDG is effectively destroyed prior to amplification.

Ordering information

Product	Quantity	Number of reactions (20 µL)	Cat. No.
SYBR® Select Master Mix for CFX			
Mini Pack	1 mL tube	100	4472937
1-Pack	5 mL bottle	500	4472942
2-Pack	2 x 5 mL bottle	1,000	4472952
5-Pack	5 x 5 mL bottle	2,500	4472953
10-Pack	10 x 5 mL bottle	5,000	4472954
Bulk Pack	50 mL bottle	5,000	4472947