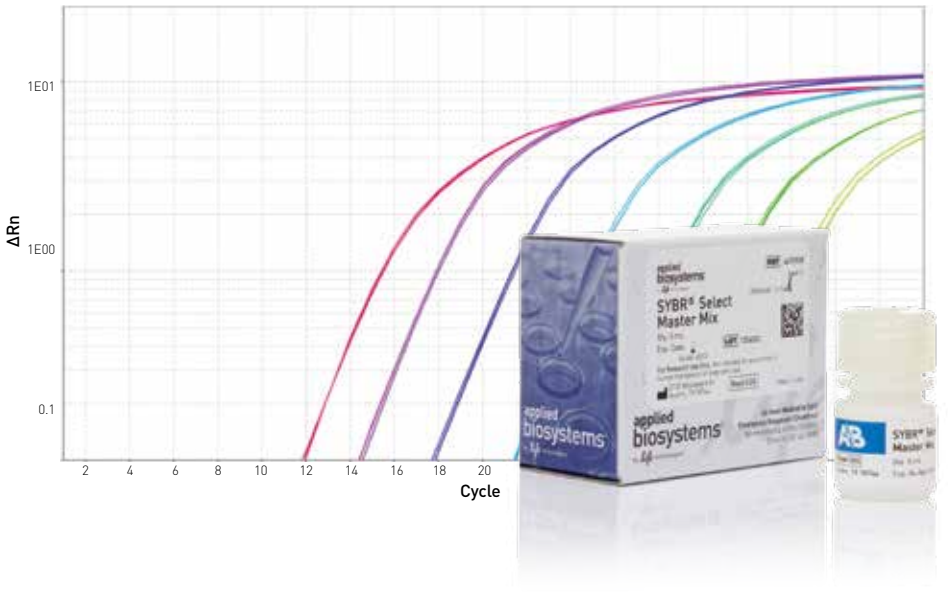
SYBR® Select Master Mix

Highly specific and sensitive quantitation

SYBR® Select Master Mix offers advanced performance at an affordable price. SYBR® Select Master Mix 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 enables highly specific and sensitive DNA, cDNA, and RNA quantitation, with true single-copy detection. SYBR® Select Master Mix 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 is formulated with SYBR® GreenER™ dye, which offers brighter signal and lower PCR inhibition than SYBR® Green I dye. In addition, SYBR® Select Master Mix 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 Applied Biosystems® real-time PCR instruments, except the 7900HT system, as well as instruments from other vendors in standard cycling mode
- Can be used in Fast mode without performance impact

life technologies

Optimized formulation for superior performance

SYBR® Select Master Mix contains all the necessary 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 offers exceptional specificity and pre-PCR benchtop stability
- SYBR® GreenER™ dye to detect double-stranded DNA—SYBR® GreenER™ dye is brighter and less inhibitory to PCR than SYBR® Green I
- Heat-labile uracil-DNA glycosylase (UDG) and a dNTP blend of dUTP/dTTP are included to help maintain optimal PCR results and help provide worry-free carryover contamination control
- Passive internal reference based on proprietary ROX™ dye, for increased precision

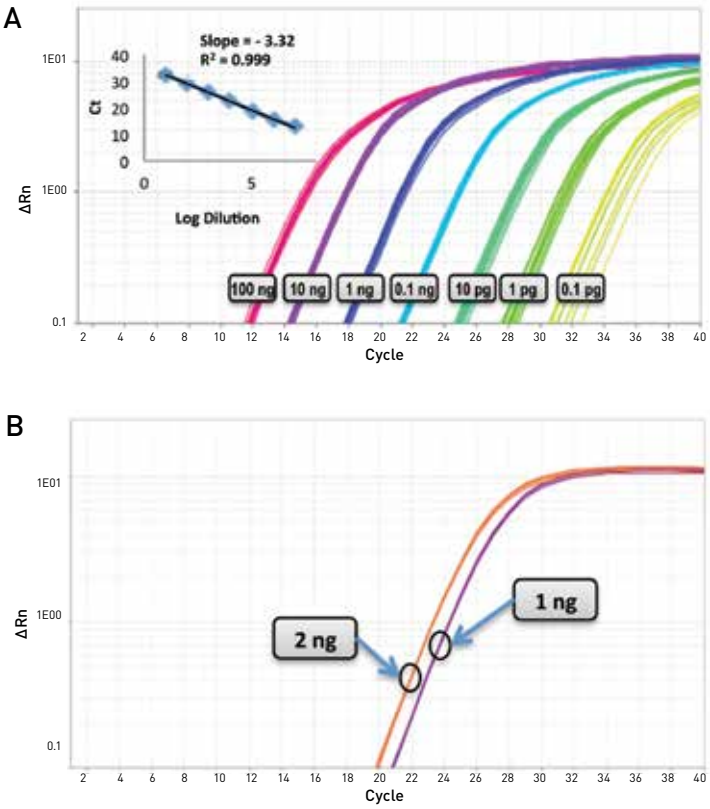


Figure 1. Use a wide range of input cDNA and obtain excellent linearity and discrimination. (A) Amplification plots and dilution curve (inset) for *PGK1* amplified from human cDNA using the ViiA™ 7 Real-Time PCR System. Up to 100 ng of input cDNA can be used without PCR inhibition or diminished PCR efficiency. **(B)** Discrimination is achieved for 1 ng vs. 2 ng cDNA input.

Reproducible results across a wide dynamic range

SYBR® Select Master Mix 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 to demonstrate the superior range and reliability of the SYBR® Select Master Mix. The amplification plot and standard curve show that the SYBR® Select Master Mix 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, 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 was tested with 44 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 44 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.

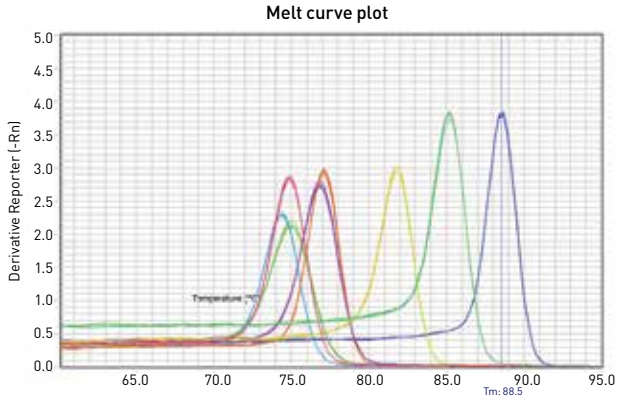


Figure 2. Representative melt curves for target genes amplified from human cDNA. Eight melt curves, with 4 replicates of each, are shown for *A2M*, *ACACA*, *AOF1*, *APOA1*, *ARL1*, β-actin, *BMP2*, and *CCNB1* assays.

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, primers, and samples. Real-time PCR was performed using the ViiA™ 7 Real-Time PCR System 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 in ΔRn), reactions containing a premix

of SYBR® Select Master Mix, primers, and sample are stable for up to 72 hours (Figure 3A). Representative amplification plots for three assays (*GAPDH*, *APOA1*, and *GUSB*) are shown for 0 hr, 24 hr, and 72 hr (Figure 3B). Melt curves obtained immediately and at 72 hours after amplification demonstrate that the PCR products are sufficiently stable to enable post-PCR analysis several days after amplification (Figure 4).

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.

The single-copy RNase P gene was amplified using genomic DNA diluted to less than 1 copy per reaction so 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 5A. Single-copy detection using SYBR® Select Master Mix was achieved, as demonstrated by the normal distribution of the C_t values and the specificity of the reactions (Figure 5B).

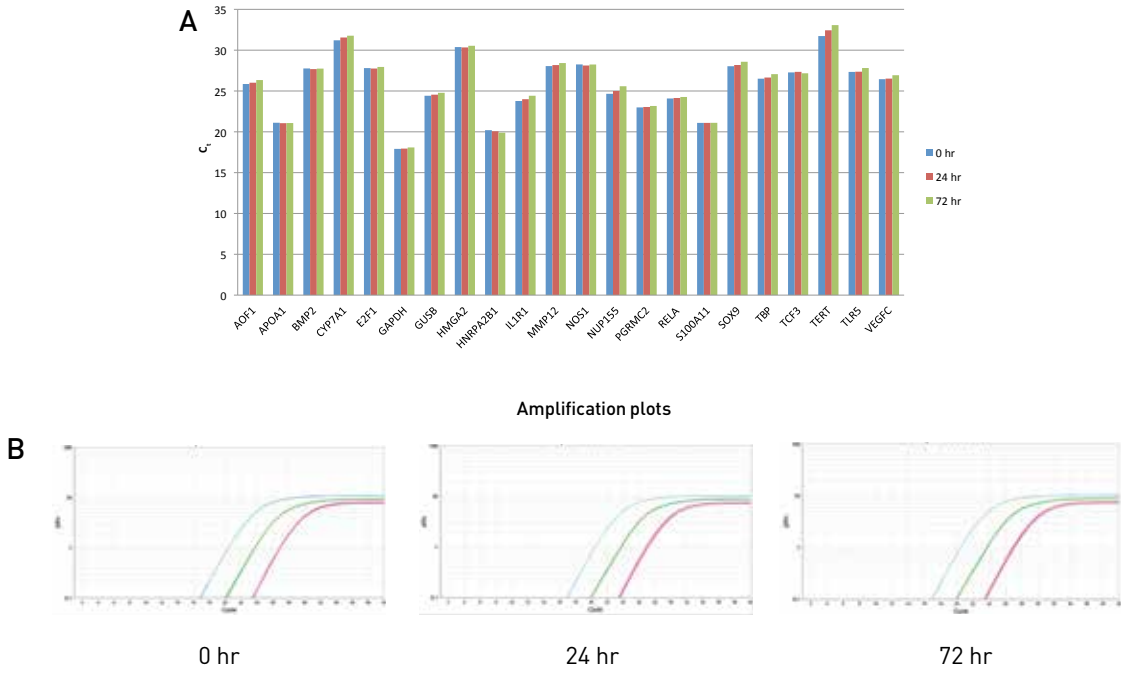


Figure 3. Pre-PCR benchtop stability. Three replicate plates were set up in parallel with SYBR® Select Master Mix, primers, and samples. Real-time PCR was performed immediately and at 24 and 72 hours after preparation. **(A)** C_t values for 22 assays. **(B)** Representative amplification plots for *GAPDH*, *APOA1*, and *GUSB*.

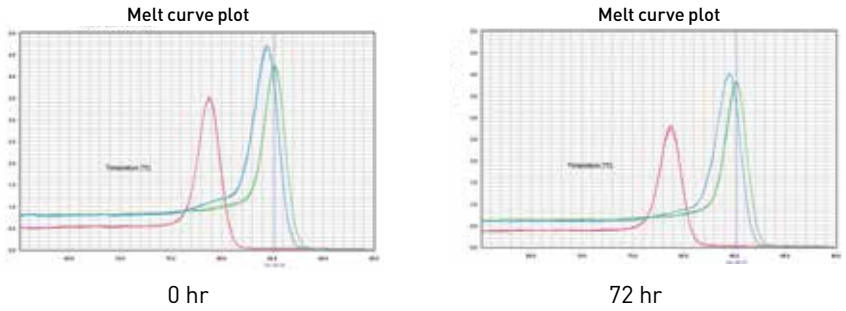


Figure 4. Post-PCR benchtop stability. Representative melt curves for *GAPDH*, *APOA1*, and *GUSB* obtained immediately and at 72 hours after amplification.

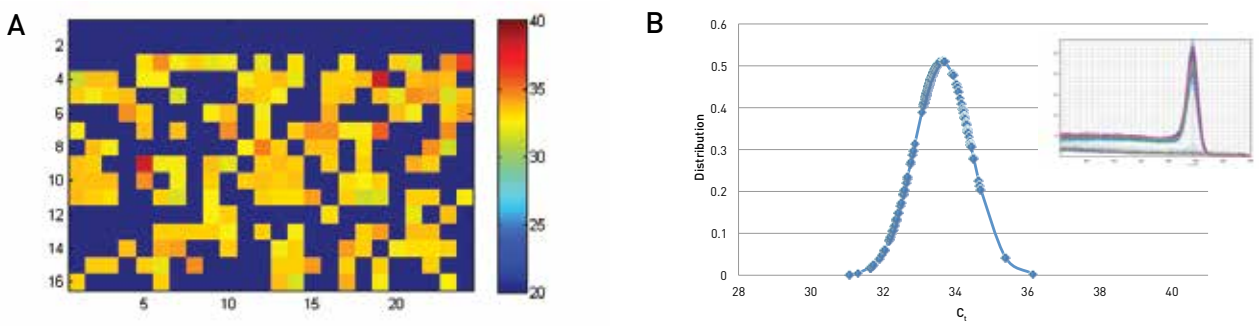


Figure 5. Single-copy detection using digital qPCR with the ViiA™ 7 Real-Time PCR System. (A) Digital PCR heat map. Genomic DNA was diluted to <1 copy per reaction to ensure that most wells would receive 1 or 0 copies of DNA. **(B)** A normal distribution of the resulting C_t values was obtained after amplification of the single-copy RNase P gene. The melt curves of all the amplified material produced a single peak (inset).

Carryover contamination control with heat-labile UDG

SYBR® Select Master Mix 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, PCR products are stable for up to 72 hours after amplification (Figure 4), demonstrating that the heat-labile UDG is effectively destroyed prior to amplification.

Broad instrument compatibility

SYBR® Select Master Mix is used in standard cycling mode and is compatible for use on all standard and Fast Applied Biosystems® Real-Time PCR instruments with 48-, 96-, and 384-well block formats, except the 7900HT Fast Real-Time PCR System. It is also compatible with the Roche® LightCycler® LC480 and Stratagene® MX3005P instruments, as well as the Bio-Rad® iQ™5, CFX96™, and CFX384™ instruments.

Fast mode for fast results—optimal primer concentration to use

The optimal primer concentration depends on the volume of the reaction and the instrument used (Table 1). For optimal performance in Standard mode, use a range of 150–400 nM for each primer. If using a StepOne™ or StepOnePlus™ Real-Time PCR System, use <200 nM of each primer.

For optimal performance in Fast mode (384-well plates), increase the primer concentration toward the higher end of the recommended range (e.g., 400 nM). If using a StepOne™ or StepOnePlus™ Real-Time PCR System in Fast mode, use 300–400 nM of each primer. Recommended cycling parameters are shown in Table 2.

Table 1. Primer concentration guide.

Mode	Primer concentration	Primer concentration for StepOne™ or StepOnePlus™ Real-Time PCR System
Standard	150–400 nM	<200 nM
Fast	400 nM	300–400 nM

Table 2. Fast mode cycling parameters.

Step	Temperature	Duration	Cycles
UDG activation	50°C	2 min	Hold
AmpliTaq® DNA Polymerase, UP activation	95°C	2 min	Hold
Denature	95°C	1 or 3 sec*	40
Anneal/extend	60°C	30 sec	

* Bio-Rad® CFX96™ Touch and CFX384™ Touch, ViiA™ 7, and QuantStudio® 12K Flex Real-Time PCR Systems: 1 sec denature; StepOne™, StepOnePlus™, and 7500 Fast Real-Time PCR Systems: 3 sec denature.

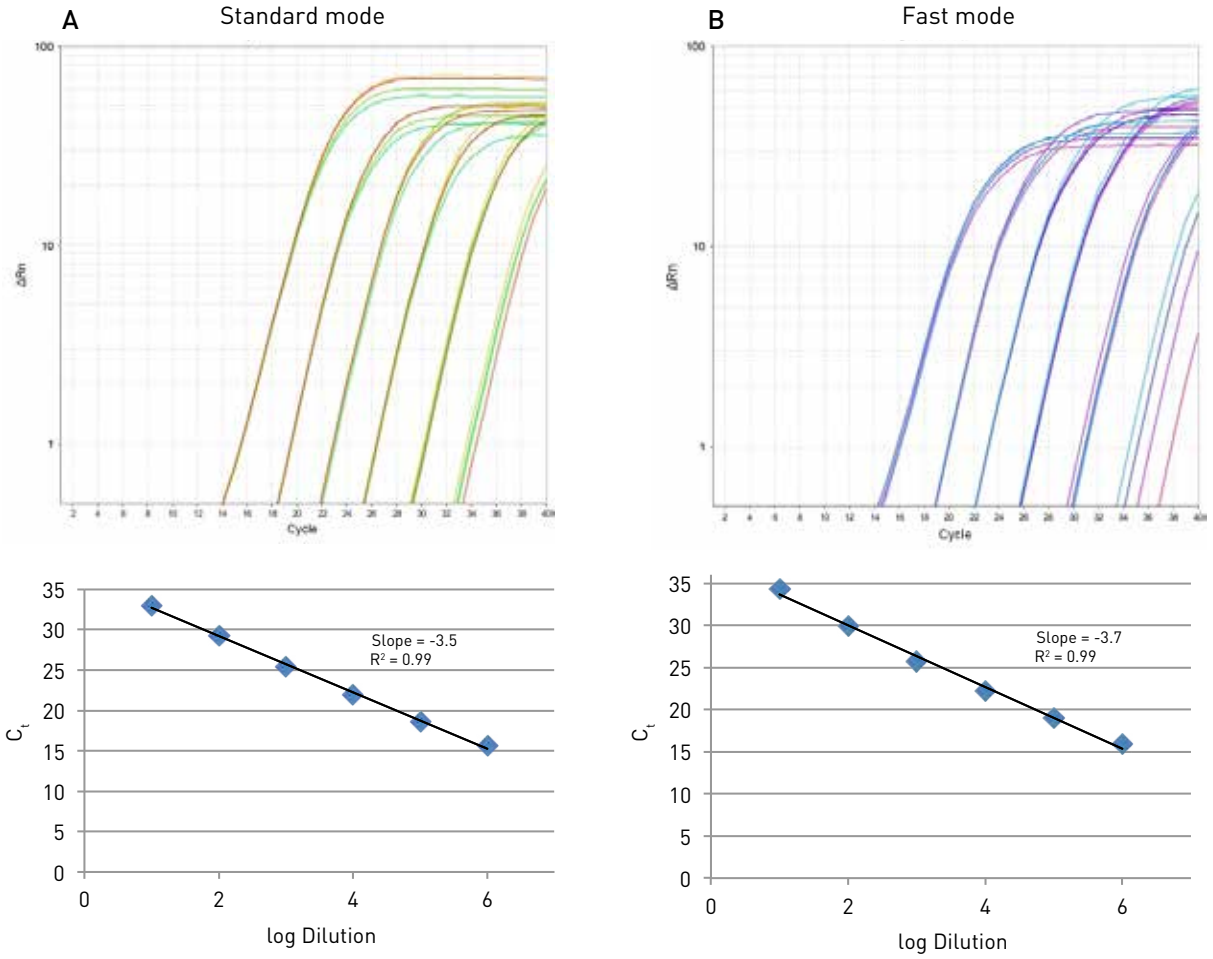


Figure 6. Amplification plots and standard curves for *PGK1* amplified from human cDNA using the ViiA™ 7 Real-Time PCR System in (A) standard mode and (B) Fast mode.

Example data

The *PGK1* gene was amplified from a 10-fold dilution series of human cDNA using the ViiA™ 7 Real-Time PCR System in both standard and Fast modes. The amplification plots and standard curves are shown in Figure 6. The C_t values are shown in Figure 7. Equivalent or nearly equivalent results were obtained for dynamic range, sensitivity, PCR efficiency, and C_t values.

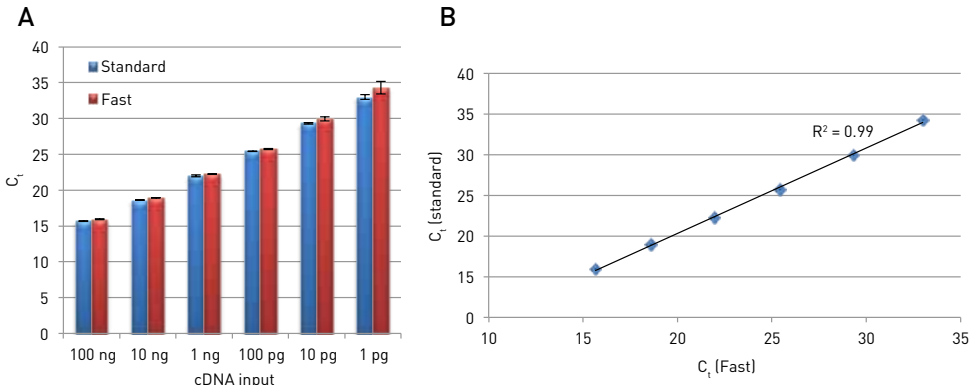


Figure 7. Comparison (A) and correlation (B) of C_t values obtained in standard and Fast modes for *PGK1* amplified from human cDNA using the ViiA™ 7 Real-Time PCR System.

Ordering information

Product	Quantity	Number of reactions (20 µL)	Cat. No.
SYBR® Select Master Mix			
Mini pack	1 mL tube	100	4472903
1-pack	5 mL bottle	500	4472908
2-pack	2 x 5 mL bottle	1,000	4472918
5-pack	5 x 5 mL bottle	2,500	4472919
10-pack	10 x 5 mL bottle	5,000	4472920
Bulk pack	50 mL bottle	5,000	4472913

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