# PowerUp SYBR Green Master Mix

## Exceptional specificity, broad instrument capability


Applied Biosystems™ PowerUp™ SYBR™ Green Master Mix is a preformulated, optimized universal 2X master mix for real-time PCR. Building on over 20 years of innovation and product excellence in qPCR, our PowerUp SYBR Green Master Mix is designed for exceptional performance in your most challenging real-time PCR applications.

### Features include:

- Exceptional specificity with dual hot-start mechanism
- Tight reproducibility in $C_t$ values over a broad dynamic range
- Compatibility with standard (results in less than 60 min) or fast (results in less than 30 min) cycling
- Formulation with uracil N-glycosylase (UNG) and dUTP to help prevent contamination by carryover PCR products
- Room-temperature stability for 72 hr after plates are prepared for qPCR
- Universal instrument compatibility

### Formulated for maximum specificity and reproducibility

PowerUp SYBR Green Master Mix contains Applied Biosystems™ Dual-Lock™ *Taq* DNA Polymerase, which has a combination of two hot-start mechanisms to control its activity. Providing tight control over *Taq* enzyme activation, this dual hot-start approach helps prevent undesirable early activity of the polymerase at low temperatures that can lead to nonspecific amplification. Formulation of the Dual-Lock *Taq* DNA Polymerase in a convenient 2X master mix containing an optimized buffer system with detergents further improves specificity of the PowerUp SYBR Green Master Mix.

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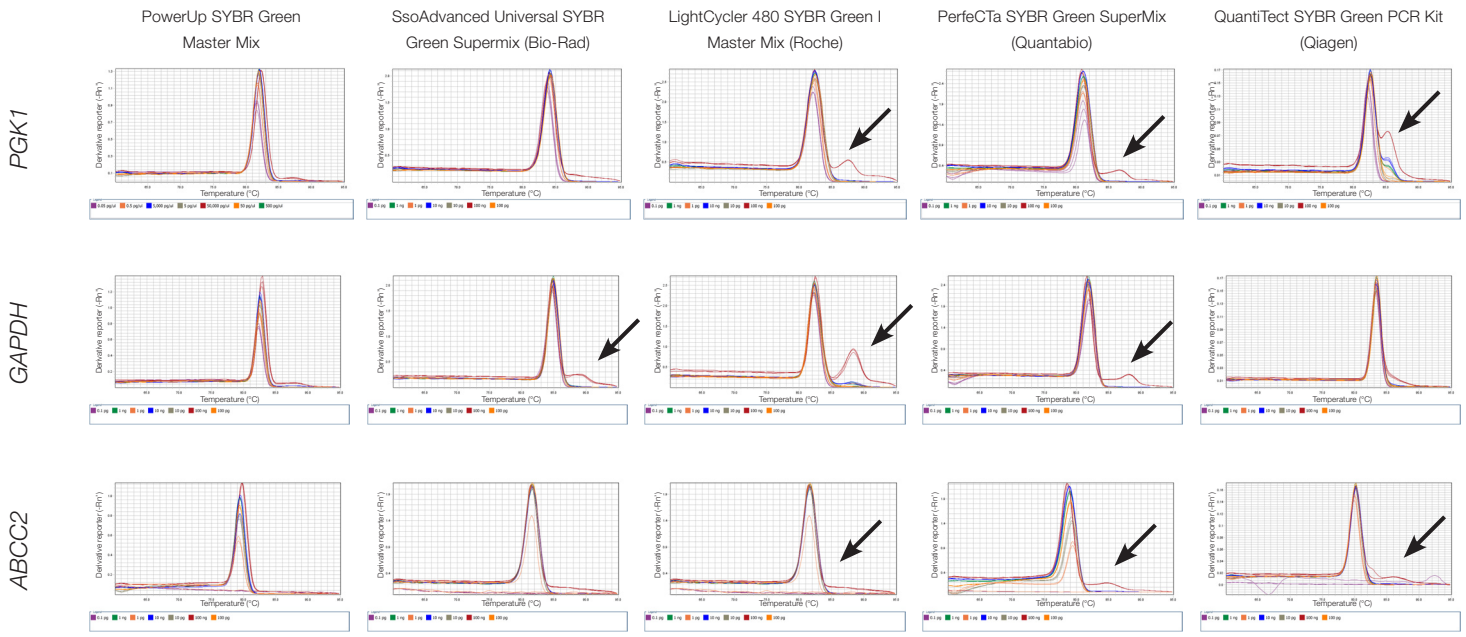


**Figure 1. Target specificity.** Melt curve analyses for 3 targets were run on the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System with 5 different master mixes. Each master mix's manufacturer's recommended cycling conditions were used with each mix on a 6-log dilution series of human universal reference cDNA.

In an evaluation of 24 different primer sets used with PowerUp SYBR Green Master Mix, a single melt curve was obtained in 100% of reactions without the need for primer optimization or redesign. In contrast, nonspecific amplification was observed for some of the same targets with several master mixes from other suppliers, as shown by multiple peaks in the melt curves (Figure 1). Validation of primer specificity in SYBR Green reactions is essential to data quality and validity [1]. The high specificity enabled by PowerUp SYBR Green Master Mix allows you to spend less time optimizing and redesigning primers to get high-quality data.

Reproducibility is another important measure of data quality in real-time PCR, and reproducibility often declines at low template concentrations, where the impacts of variability are exacerbated. However, PowerUp SYBR Green Master Mix, with its dual hot-start *Taq* DNA polymerase, demonstrates excellent reproducibility over a wide dynamic range with a variety of targets tested (Figure 2). Tighter reproducibility allows for greater statistical significance when analyzing low-abundance transcripts and smaller fold changes.

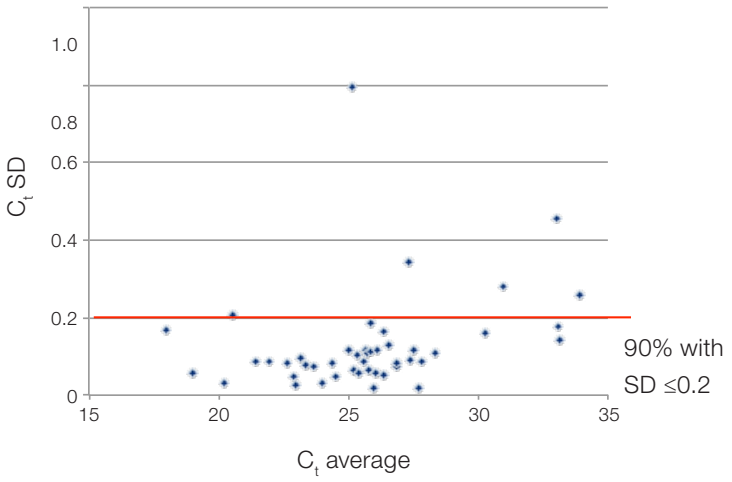


**Figure 2. Reproducibility of data.** Quadruplicate reactions were run for 24 assays with primer concentrations of 300 nM on two lots of PowerUp SYBR Green Master Mix on the Applied Biosystems™ 7900HT system. The standard deviation (SD) of the $C_t$ is plotted against the average $C_t$ for the quadruplicate reactions for each assay for each lot. Average $C_t$ values greater than 34 were excluded from this analysis.

## Enabling reliable results across a wide dynamic range

PowerUp SYBR Green Master Mix provides robust results with 0.1 pg to 100 ng cDNA per reaction.* Generating consistent amplification across a wide dynamic range is fundamental to qPCR methodology, including utilization of the ΔΔCt method for gene expression analysis. Unlike master mixes from other suppliers that can exhibit inhibition at high cDNA inputs and low correlation coefficients over a broad dynamic range, PowerUp SYBR Green Master Mix efficiency is maintained over 6 logs of input to enable maximum confidence in data quality (Figure 3, Table 1).

## UNG for carryover contamination control

Contamination in labs that routinely run PCR is a major concern for many researchers and is the source of most false positives. The inclusion of UNG and dUTP in the PowerUp SYBR Green Master Mix degrades any previously amplified PCR products and helps prevent contamination of subsequent qPCR reactions. Utilization of a heat-labile UNG enzyme allows for effective contamination control within the PowerUp SYBR Green Master Mix without impacting downstream analysis such as melt curve or gel analysis of the PCR product.

**Table 1. Comparison of dynamic ranges of various suppliers' master mixes.** The numbers indicate the linear range that can be measured by each mix for each target gene. For example, "6" means 6 orders of magnitude (logarithmic units), spanning seven 10-fold dilutions from 100 ng to 0.1 pg of universal human reference cDNA per 10 µL reaction.

| Reagent | *GAPDH* | *ABCC2* | *ARL1* | *PGK1* |
|---|---|---|---|---|
| PowerUp SYBR Green Master Mix | 5 | 5 | 4 | 6 |
| Bio-Rad mix 1 | 5 | 3 | 4 | 4 |
| Bio-Rad mix 2 | 5 | 3 | 4 | 4 |
| Roche mix | 5 | 3 | 2 | 4 |
| Quantabio mix | 5 | 4 | 4 | 4 |
| Qiagen mix | 5 | 3 | 3 | 4 |

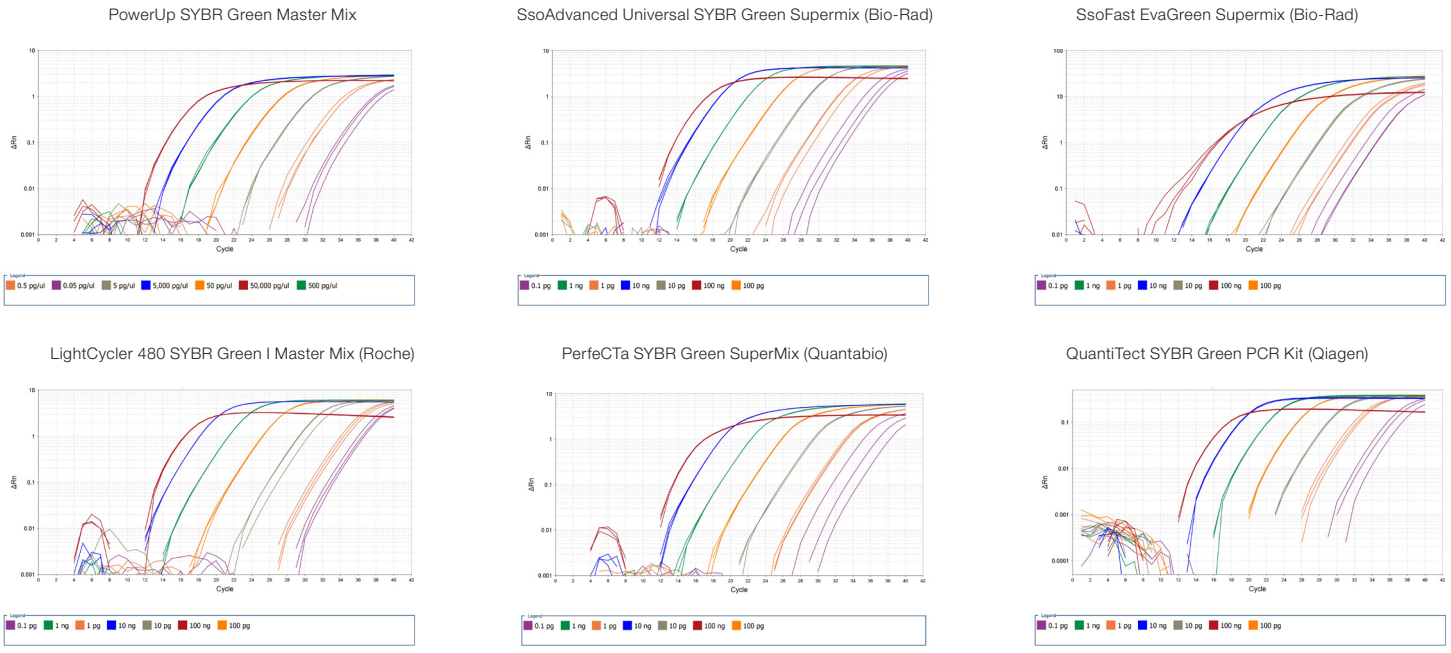


**Figure 3. Robust performance of PowerUp SYBR Green Master Mix over a broad dynamic range.** Amplification curves were obtained for the *ARL1* gene over a 6-log dilution series of human universal reference cDNA. Reactions were run in triplicate on the QuantStudio 7 Flex Real-Time PCR System according to each master mix's manufacturer's recommended protocol. Master mixes from other suppliers resulted in greater inhibition, as demonstrated by the shape of the curves and the narrowed spacing between them at the highest cDNA inputs. In addition, several mixes from other suppliers failed to reliably detect the lowest dilution. *ARL1* amplification was confirmed to produce single melt curves for each master mix (data not shown).

* Besides the master mix, other assay conditions and reagent concentrations may affect dynamic range; individual results may vary.

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## Broad instrument compatibility

PowerUp SYBR Green Master Mix can be used in either standard or fast cycling mode and is compatible with all Applied Biosystems™ real-time PCR instruments.† It is also compatible with the Bio-Rad CFX96™, CFX384™, and iQ™5, Roche LightCycler™ 480, and Agilent Mx3005P™ systems. For optimal results, primer concentrations should be in the range of 300–800 nM.

## Preassembled reactions stable after 72 hours

PowerUp SYBR Green Master Mix, with its Dual-Lock *Taq* DNA Polymerase, is designed to generate consistent performance in reactions preassembled and held for up to 72 hours before running them (Table 2). One factor that helps improve benchtop stability is that the dye in this master mix is less light-sensitive than other SYBR™ Green dyes. The stability of this master mix provides users with the flexibility to set up plates prior to cycling without impacting results.‡

**Table 2. Pre-PCR reaction stability.** Several targets were amplified using PowerUp SYBR Green Master Mix and 1 ng human cDNA on an Applied Biosystems™ StepOnePlus™ Real-Time PCR System, either immediately following reaction setup or after 72 hours of incubation at room temperature in the dark. Single melt curves were confirmed for these assays at both the 0 hr and 72 hr time points.

| Target gene | $C_t$, 0 hr | $C_t$, 72 hr | $\Delta C_t$ |
|---|---|---|---|
| *ADAM10* | 25.2 | 25.6 | 0.4 |
| *APOA1* | 24.9 | 24.9 | 0.0 |
| *CCNB1* | 26.0 | 26.1 | 0.1 |
| *COL1A1* | 23.9 | 23.9 | 0.0 |
| *CTGF* | 24.3 | 24.7 | 0.4 |
| *METTL3* | 26.3 | 26.7 | 0.4 |


### Reference

1. Bustin SA, Benes V, Garson JA et al. (2009) The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem* 55:611–622.


### Ordering information

| Product | | | |
|---|---|---|---|
| **PowerUp SYBR Green Master Mix** | **Quantity** | **Number of reactions (20 µL)** | **Cat. No.** |
| Mini pack | 1 mL | 100 | A25741 |
| 2-pack | 2 x 1 mL | 200 | A25779 |
| 1-pack | 1 x 5 mL | 500 | A25742 |
| 5-pack | 5 x 1 mL | 500 | A25780 |
| 2-pack | 2 x 5 mL | 1,000 | A25776 |
| 10-pack | 10 x 1 mL | 1,000 | A25918 |
| 5-pack | 5 x 5 mL | 2,500 | A25777 |
| Bulk pack | 1 x 50 mL | 5,000 | A25743 |
| 10-pack | 10 x 5 mL | 5,000 | A25778 |

† Recommend fast or standard cycling on all instruments except the Applied Biosystems 7900HT system, for which we recommend standard cycling.

‡ Pre-PCR stability is influenced not only by the master mix but by the target being analyzed as well. For maximum confidence, researchers should confirm stability profiles of their specific targets.

Find out more at **thermofisher.com/sybr**

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